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Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.01/A1

Topic: A.01. Brain Patterning

Support: Swiss National Science Foundation (Sinergia CRSI33_127440)

Novartis Research Foundation

Title: Epigenetic control of Hox paralogue group 5 induction in tangentially migrating precerebellar neurons

Authors: D. KRAUS[1], T. DI MEGLIO[1], C. F. KRATOCHWIL[1], S. DUCRET[1], *F. M. RIJLI[2]; [1]Friedrich Miescher Inst. for Biomed Res., Basel, Switzerland; [2]Friedrich Miescher Inst., Basel, Switzerland

Abstract: In mammals, cortical motor and sensory information is mostly relayed to the cerebellum via the hindbrain precerebellar pontine nuclei (PN), which include pontine gray and reticulotegmental nuclei. The developing hindbrain is rostrocaudally segregated into progenitor compartments, or rhombomeres (r1-r8), genetically defined by nested Hox gene expression. Mouse PN neurons are generated from r6-r8 lower rhombic lip (lRL) progenitors, undergo a long-distance caudorostral tangential migration via the anterior extramural stream (AES), and settle beside the ventral midline. Intrinsic expression of transcription factors and guidance receptors and extrinsic distribution of ligands are important for AES migration. We have recently shown that the AES has an intrinsic topographic organization (Di Meglio et al, Science 339, 204-7, 2013). Namely, the position and migratory path of distinct subsets of PN neurons within the AES is defined by their relative rostrocaudal origin in the progenitor compartment and nested Hox gene expression is mantained throughout migration and nucleogenesis.
Here, we investigated the molecular mechanisms governing the precise spatial and temporal regulation of Hox expression in AES neuron subsets. We found that, unlike other Hox genes, the expression of Hox PG5 genes is absent in lRL progenitor cells. We show that high levels of locally produced retinoic acid (RA) are in turn required to overcome an Ezh2-mediated repression and induce Hox PG5 expression at the onset of tangential migration in a specific subset of late-born neurons targeting the posterior PN.

Disclosures: D. Kraus: None. **F.M. Rijli:** None. **T. Di Meglio:** None. **C.F. Kratochwil:** None. **S. Ducret:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.02/A2

Topic: A.01. Brain Patterning

Support: Klingenstein Foundation

Basil O'Connor Award, March of Dimes

NSF 1120796

NIH-NINDS R01NS073055

Shriners Hospital Research Grant

Shriners Hospital Postdoctoral Fellowship

Title: Glutamate signaling during neural tube formation

Authors: *P. A. CASTRO[1], E. B. SEQUERRA[1], L. TIAN[2], L. N. BORODINSKY[1];
[1]Dept. of Physiol. & Membrane Biol., [2]Dept. of Biochem., Univ. of California Davis Sch. of Med., Sacramento, CA

Abstract: One of the earliest events in the development of the vertebrate nervous system is the process of neurulation, when the folding of the neural plate is followed by the closure of the neural tube. The failure of neural tube formation leads to one of the most common birth defects known as neural tube defects. Antiepileptic drug use during pregnancy increases the incidence of neural tube defects in offspring by unknown mechanisms. Our previous studies have shown that glutamate signaling through NMDA receptors is important for the formation of the neural tube and suggest that antiepileptic drugs may alter neurotransmitter signaling during neurulation. In this study we investigate the molecular mechanisms by which glutamate is released and signals in the folding neural plate.

We assessed the expression of molecular components of neurotransmitter, SNARE complex-dependent, vesicular release and found by reverse transcriptase-PCR that transcripts of synaptobrevin, syntaxin, SNAP25 and synaptotagmin are all present in neural plate tissue of Xenopus laevis embryos. Another potential mechanism for neurotransmitter release is through the connexin-formed hemichannels. We detected transcripts for the connexins Cx43, Cx38, Cx46, Cx32 and Cx26 at these early developmental stages. In addition, transcripts of glutamate receptor subunits, GluR1 (AMPAR), GluR7 (KAR) and NR1 (NMDAR) are present in the

folding neural plate.
In order to determine the source of glutamate and the dynamics and mechanisms of its release during neural plate folding we expressed the membrane-anchored, extracellular glutamate-sensing fluorescent reporter, iGluSnFR, in developing Xenopus embryos by microinjecting the mRNA in one- and two-cell stage embryos. This approach renders a uniform protein expression in the whole embryo. In contrast, in vivo imaging of neurulating embryos reveals that the basal green fluorescence intensity is 3-fold higher in the dorsomedial region of the embryo, compared to dorsolateral tissue. This signal is diminished by 20% in the presence of the enzyme glutamate-pyruvate transaminase, which deaminates glutamate in the presence of pyruvate, decreasing available glutamate for binding to the probe. On the other hand, exogenous addition of 0.5-2 mM glutamate enhances the fluorescence intensity by 25% in the folding neural plate. These preliminary results suggest that the glutamate sensor reports in vivo dynamics of extracellular glutamate due to the release of glutamate in the folding neural plate.
Current efforts are focused on determining the mechanisms of glutamate release during neural tube formation.

Disclosures: P.A. Castro: None. **E.B. Sequerra:** None. **L. Tian:** None. **L.N. Borodinsky:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.03/A3

Topic: A.01. Brain Patterning

Support: Autism Speaks! 2007/2552

NIH/NICHD P50-HD-055784

Title: Anatomical impact of reelin haploinsufficiency and prenatal organophosphate pesticide exposure in mouse cerebellum

Authors: B. ROSS, B. MULLEN, *E. M. CARPENTER;
Dept. of Psychiatry and Biobehavioral Sci., UCLA Sch. Med., Los Angeles, CA

Abstract: Autism Spectrum Disorders (ASDs) are human developmental disorders characterized by a lack of innate social skills, impaired communication, reduced emotional response, and compulsive repetitious behaviors. ASDs are likely caused by a combination of genetic and environmental factors. In this study, we examined interactions between two possible contributing

factors, reduced expression of the reelin gene and prenatal exposure to an organophosphate pesticide. The reduced protein and mRNA levels in ASD patients suggest reelin is a candidate gene for ASD, and exposure to organophosphate pesticides has been linked to a higher incidence of autism in rural areas. Despite extensive study, few consistent anatomical changes have been observed in ASD patients. In this study, we specifically examined the organization and distribution of Purkinje cells in the cerebellum. Prior studies have shown a reduced Purkinje cell population in ASD patients, and loss of reelin expression has been shown to affect Purkinje cell death in reelin-deficient mice. Previous findings also show that the organophosphate pesticide CPO can also decrease the number of Purkinje cells. This study tested whether there will be an additive effect that would cause a more severe reduction of Purkinje cells in CPO-treated reelin-deficient mice than in either reelin-deficient or CPO treatment alone. Preliminary results suggest a loss of Purkinje cells and alterations in cerebellar foliation in CPO-treated reelin-deficient mice. Understanding the genetic and environmental influences that contribute to ASDs could culminate in preventative measures to reduce the prevalence or severity of ASDs in afflicted individuals.

Disclosures: B. Ross: None. **B. Mullen:** None. **E.M. Carpenter:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.04/A4

Topic: A.01. Brain Patterning

Support: Ministry of Education, Science and Technology (2010-0010803)

Title: Ciliary length control and Hedgehog signaling in mouse development

Authors: J. SONG, H.-J. MOON, S. LEE, H. LEE, *H. KO;
Col. of Pharm., Dongguk Univ., Goyangsi, Korea, Republic of

Abstract: Most of mammalian cells including neuronal cells maintain single hair-like structures on the surface of extracellular membrane known as primary cilia. Physiological importance of primary cilia in mammals remains enigmatic for past century and recent advance in cilia biology research enlightened the crucial roles of primary cilia in animal development and disease. Although primary cilia are involved in multiple key developmental signaling pathways, Hedgehog (Hh) signaling pathway is best known and tightly regulated by primary cilia. Previously our lab identified the novel Hh signaling pathway component, Bromi, and loss of its expression *in vivo* causes dorsalized neural tube and diminished Hh signaling activity in mouse

neural tube development. To better understand the function of Bromi in neural tube development, we identified Bromi interacting protein, Cell cycle related kinase (CCRK), which is a homologue of long flagellar mutant 2 (LF2) in green algae, *Chlamydomonas*. The model system of *Chlamydomonas* has provided precious insight of mechanisms of ciliogenesis in mammalian system. Using this simplified model system, it has been isolated 4 different genes regulating flagellar length. We are interested in how ciliary length control impacts on transducing Hh signaling in mouse development. We generated KO mice for mouse homologue of LF gene and analyzed the developmental defects in LF gene KO mice. We observed that downregulation of mouse homologue of LF gene expression caused diminished Hh signaling and abnormal ciliogenesis. Multiple developmental organogenesis defects were manifested in mutant mice with misregulation of Hh signaling. Based on our findings, proper length of primary cilia throughout vertebrate development is critical for ensuring the proper animal development and homeostasis.

Disclosures: J. Song: None. **H. Ko:** None. **H. Moon:** None. **S. Lee:** None. **H. Lee:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.05/A5

Topic: A.01. Brain Patterning

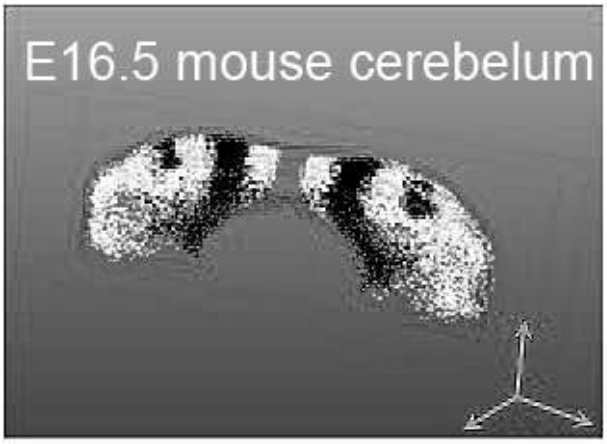
Support: Grant-in-Aid for Scientific Research (C) 25430008

Title: Formation process of the cerebellar compartments that are determined by birthdate of Purkinje cells

Authors: *M. HASHIMOTO, K. YAMADA, T. MIYATA;
Aichi, Nagoya Univ. Med. Cell Biol., Nagoya, Japan

Abstract: The adult cerebellar cortex is organized into longitudinal compartments that are revealed by specific axonal projections (olivocerebellar and corticonuclear projections). The cerebellar compartments are thought to be the basic and functional subdivisions of the cerebellum. Using an adenoviral vector, we label Purkinje cells (PCs) born at embryonic day (E) 10.5, E11.5, and E12.5 and trace their fated positions in the mouse cerebellum. As a result, each cohort of birthdate-related PCs forms longitudinal compartments in the cerebellum, and furthermore, these compartments correlate strikingly with the compartments revealed by olivocerebellar projections. It suggests that there is close correlation between birthdate of PCs and the formation of cerebellar neuronal network. However, the formation process of the

cerebellar compartments has not been elucidated.
Using an adenoviral vector expressing enhanced yellow fluorescence protein, fluorescence-activated cell sorting, and DNA microarray analysis, we defined the genetic properties of E10.5-, E11.5-, and E12.5-born PCs. As a result, each cohort of birthdate-related PCs was characterized by the expression of a subset of particular genes, which was involved in regional specificity (e.g., EphA7) and neuronal function (e.g., EAAT4) in the cerebellum. Furthermore, we identified marker proteins for E10.5-born PCs and E11.5-born PCs. Using specific antibodies against these markers, we examined the distribution of E10.5-born PCs and E11.5-born PCs in the embryonic mouse cerebellum. 3D reconstruction of these distributions revealed the formation process of the cerebellar compartments in mouse cerebellum. E10.5-born PCs and E11.5-born PCs individually formed layers at E13.5 and these layers changed drastically into compartments after E14.5.



Disclosures: M. Hashimoto: None. **K. Yamada:** None. **T. Miyata:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.06/A6

Topic: A.01. Brain Patterning

Title: Role of galanin in neuronal cell migration after brain injury

Authors: *H. KOMURO, J. FAHRION;
Lerner Res. Inst. Cleveland Clin., CLEVELAND, OH

Abstract: During the prenatal, perinatal, and postnatal periods, brains are very vulnerable to insults (such as traumatic injury and ischemia), which can cause permanent defects in neuronal

cytoarchitecture and lead to motor, cognitive, and learning deficits in children. To date, little is known about the mechanisms by which insults cause disruption of brain development. Because we thought that the neuropeptide galanin might be involved in the insult-induced disruption of brain development, we examined the role of galanin in the cerebellar growth of early postnatal mice after insults induced by freezing treatment (FT). In this study, to induce focal injury on the center of lobule VIb of postnatal (P) 10-day-old mouse cerebellum, a cooled (-70^{o}) 1 mm-diameter platinum probe was placed on the midline of the occipital bone (OcB) near the border between the OcB and interparietal bone for 10 seconds. In control animals, the probe, which was maintained at room temperature, was placed on the same site of the OcB. Here we show that galanin plays opposite and region-specific roles in the FT-induced defects in cerebellar cortical layer development through alterations in granule cell migration. First, FT caused decreased growth of cerebellar cortical layers in the region (lobule VIb) under the skull where the cooled probe was placed, but increased growth in the surrounding region (crus II). Second, FT decelerated granule cell migration in the frozen region (lobule VIb) where cortical layers became thinner, but accelerated the migration in the surrounding regions (crus II) where cortical layers became thicker. Third, FT increased galanin levels in regions (lobule VIb) where granule cell migration accelerates, but decreases galanin levels in regions (crus II) where granule cell migration decelerates. Fourth, *in vitro* studies revealed that the application of exogenous galanin accelerates granule cell migration in the EGL, ML, and IGL by altering Ca^{2+} and cAMP signaling, and Ca^{2+} transients via the activation of galanin receptors. Fifth, in vivo studies demonstrated that the injection of galanin or galanin receptor inhibitor can ameliorate the effects of FT on granule cell migration and cortical layer growth in both the frozen regions (lobule VIb) and surrounding regions (crus II). These results indicated that modification of galanin signaling provides crucial cues for restoring brain development after injury.

Disclosures: H. Komuro: None. **J. Fahrion:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.07/A7

Topic: A.01. Brain Patterning

Title: Chondroitin sulfotransferase expression pattern in migrating motor neurons of the rat embryonic hindbrain

Authors: *M. WONG[1], M. LI[1], Y. S. CHAN[2], D. K. Y. SHUM[1];
[1]Biochem., [2]Physiol., The Univ. of Hong Kong, Hong Kong, China

Abstract: Chondroitin sulfate (CS) moieties of proteoglycans are extracellular matrix components that have been implicated in the timing and patterning of axon fasciculation during embryonic brain development. It, nonetheless, remains unclear if these moieties can also control neuronal soma migration in the developmental process. We hypothesized that the cranial motor neuron migration in the hindbrain is modulated by varying sulfation patterns of the chondroitins between the migrating and ready-to-migrate neurons. In this project, hindbrain explants of E11.5 Sprague Dawley rats were maintained in culture. In control cultures, time lapse video microscopy revealed advancement of neuronal cell bodies in the direction of the leading process away from the explant core. In test cultures treated with chondroitinase ABC, the neuronal cell bodies lost the directional movement but not the motility. Immunocytochemistry confirmed the presence of CS56 epitopes among Tuj-1-positive neurons not only in the explant core and those advancing beyond the core, but also in the environment surrounding the migrating neuronal cell bodies. In situ hybridization revealed the relatively abundant expression of chondroitin-4-sulfotransferase 2 (C4ST2) mRNA among cells heading away from the explant core. Taken together, the present results showed that CS moieties expressed by migrating neurons differ in sulfation pattern from those in the vicinity of non-migrating neurons.

Disclosures: M. Wong: None. **M. Li:** None. **Y.S. Chan:** None. **D.K.Y. Shum:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

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Program#/Poster#: 219.08/A8

Topic: A.01. Brain Patterning

Support: Norwegian Research Council Grant 196122

Title: Neurodevelopmental toxicity caused by prenatal exposure to bisphenol A and di-isononyle phthalate

Authors: *K. E. RAKKESTAD, G. H. MATHISEN, M.-C. DELEBECQUE, R. E. PAULSEN; Univ. of Oslo, Sch. of Pharm., OSLO, Norway

Abstract: Environmental chemicals like bisphenol A (BPA) and phthalates may cause neurodevelopmental damage, and special concern is expressed for the neurotoxic effects on the developing nervous system of fetuses, babies and children. These compunds cross the placenta as well as the blood-brain-barrier. We use *in ovo* exposure of chicken as a model for in utero and neonatal exposure. In the cerebellum, tight regulation of the level of the transcription factor Pax6 is critical for correct development of granule neurons. We have exposed chicken embryos at

ED16 to BPA (~0.23 µg/g egg) for 24 hours before preparation of cerebellar granule cell cultures, and measured the Pax6 level on day 3 and 6 *in vitro*. We have also investigated whether prenatal exposure of mice pups to BPA induced changes in the Pax6 level in their granule neurons. These mice pups were obtained from mothers receiving BPA in the drinking water before mating, during pregnancy and during lactation. The Pax6 level was measured in the whole cerebellum from pups of various ages. Also, possible effects of prenatal exposure to BPA on the morphology of the cerebellum were investigated by measuring the thickness of the different layers in H&E stained cerebellar sections. In cultured chicken cerebellar granule neurons from BPA injected eggs we found that the Pax6 level was increased at day 6 *in vitro*. Further, we found that BPA induced an increase in the thickness of the external granule layer and also an increase in the total cerebellar Pax6 level in 11 days old mice offspring. The chicken model was also used for studies of prenatal di-isononyle phthalate (DINP) exposure. We injected solutions resulting in final concentrations inside the eggs of approximately 10nM, 100nM and 1µM. Preliminary results indicate that exposure to DINP *in ovo* reduces the Pax6 level measured in whole cerebella. Together these findings indicate that both bisphenol A and di-isononyle phthalate may affect neurodevelopment in the cerebellum.
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Disclosures: K.E. Rakkestad: None. **G.H. Mathisen:** None. **M. Delebecque:** None. **R.E. Paulsen:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.09/A9

Topic: A.01. Brain Patterning

Title: Activation of Hedgehog signaling pathway in the developing neural retina stimulates proliferation and disrupts optic fissure closure

Authors: *K. SAKAGAMI, X.-J. YANG;
Ophthalmology, UCLA, Jules Stein Eye Inst., LOS ANGELES, CA

Abstract: The mature vertebrate neural retina originates from the anterior neural plate and consists of seven major types of neuronal and glial cells. Accumulating evidence indicates that proliferation and cell fate specification of neural progenitor cells are regulated by both cell-intrinsic factors and cell-extrinsic cues. Among the known cell-extrinsic cues, the Hedgehog (Hh) family of molecules has been shown to regulate neural tissue patterning, cell proliferation,

laminar organization, and neuronal differentiation in the vertebrate retina. Previously, we have reported that conditional knockout of Smoothened (Smo), a receptor component of Hedgehog signaling, resulting in reduction of progenitor cell pool and increased retinal ganglion cell production in the embryonic retina. For gain-of-function experiments of Hh signaling, Rosa26-SmoM2 (R26-SmoM2) mice were crossed with Chx10-Cre driver mice. SmoM2 activation mutant shows enhanced proliferation and abnormal differentiation accompanied with increase of Sox2- and Sox9-positive progenitor cells in the embryonic retina. Immunohistochemistry also revealed increase of Pax2-positive cells in ventral retina and disrupted optic fissure closure, which mimics human coloboma. Current investigation is focused on understanding mechanisms of Hh signaling regulated eye morphogenesis and retinal development, and how disruption of Hh signaling leads to a congenital eye disease.

Disclosures: K. Sakagami: None. **X. Yang:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.10/A10

Topic: A.01. Brain Patterning

Support: NICHD Grant HD062499

Title: The Gene Expression Database (GXD) for mouse development

Authors: *J. H. FINGER, T. F. HAYAMIZU, I. J. MCCRIGHT, C. M. SMITH, J. XU, J. T. EPPIG, J. A. KADIN, J. E. RICHARDSON, M. RINGWALD;
The Jackson Lab., BAR HARBOR, ME

Abstract: The Gene Expression Database (GXD) provides neuroscientists with a unique expression resource, where results and images from many assay types, anatomical structures, and developmental stages can be easily searched and analyzed. A free resource of mouse developmental expression data, GXD integrates results from RNA in situ hybridization, immunohistochemistry, knock-in reporter, RT-PCR, northern, and western blot experiments. Curators read the literature and enter the expression data therein. They also work with individual scientists and large-scale data providers to incorporate electronic submissions into the database. GXD includes over 1.3 million annotated results, more than 248,000 expression images, and data from 1,800 mouse mutants. Data are recorded and integrated by using standard gene and allele nomenclature, a hierarchical developmental stage-specific anatomy ontology, and a variety of controlled vocabularies. This makes it easy to search, navigate and compare the expression data.

Search parameters include gene or gene sets [defined by Gene Ontology (GO), phenotype, or disease], anatomical structure, developmental stage and assay type. GXD is part of the larger Mouse Genome Informatics (MGI) resource (www.informatics.jax.org), so scientists can explore the relevance of expression data through its integration with genetic, genomic, biological, and phenotypic information.
Recently, we have significantly enhanced the query and display capabilities of GXD. We have added new search functions to the Gene Expression Data Query form, and made the search form easier to use through an improved layout and other utilities. We have improved query performance so that even large sets of data can be returned quickly. Each search returns a summary that provides multiple views (via tabs) of the results, images, and genes. Most columns on these summaries are sortable, and export features have been added to download the data in text and spreadsheet formats. The summaries lead to enhanced assay detail pages that now include images alongside corresponding text annotations of the results.Visit the GXD homepage at www.informatics.jax.org/expression.shtml.

Disclosures: J.H. Finger: None. **T.F. Hayamizu:** None. **I.J. McCright:** None. **C.M. Smith:** None. **J. Xu:** None. **J.T. Eppig:** None. **J.A. Kadin:** None. **J.E. Richardson:** None. **M. Ringwald:** None.

Poster

219. **Patterning of Spinal Cord, Brain Stem, and Cerebellum**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 219.11/A11

Topic: A.01. Brain Patterning

Support: NIH/NINDS

Title: Bone Morphogenetic Proteins (BMPs) regulate neural tube closure by cell cycle dependent mechanisms: A 4D time-lapse analysis

Authors: C. Y. BROWN[1], D. EOM[3], *S. AGARWALA[2];
[1]Inst. for Cell and Mol. Biol., [2]Mol. Cell and Developmental Biol., Univ. Texas at Austin, AUSTIN, TX; [3]Dept of Biol., Univ. of Washington, Seattle, WA

Abstract: During neural tube closure (NTC), a flat neural plate rolls up and closes to form a neural tube. This process depends upon the formation of a ventral midline hinge point (MHP), which buckles the neural plate and elevates the neural folds. MHP formation is marked by dynamic cell-shape changes, which result in wedge-shaped cells displaying increased basal: apical width ratios. Multiple models of cell wedging at the MHP have been put forward. These

include constriction of the apical adherens belt via cytoskeletal and junctional remodeling or via polarized endocytosis. These models do not fully explain how cell wedging occurs in the amniote where a single layer of bipolar progenitors spans the apicobasal axis/ventricular pial axis of the neural plate. These cells undergo interkinetic nuclear migration as they progress through the cell cycle, with large M phase cells occupying the apical surface and interphase (G1, G2, S) nuclei occupying the thickness of the neural plate. Cell wedging in such a tissue may simply involve keeping nuclei at basal locations via cell cycle dependent mechanisms. We have recently provided evidence that the blockade of Bone Morphogenetic Proteins (BMP) plays a critical role in MHP induction, where it induces basal nuclear retention or migration. Although we have provided evidence for BMP-mediated regulation of tight and adherens junctions, its role in regulating cell cycle kinetics has not been explored.
Here, we examined BMP-cell cycle interactions by combining in vivo PCNA-GFP and BMP manipulations with time-lapse analyses in 3-D midbrain explants.
We show that the cell cycle length is increased at ectopic hinges induced by BMP blockade by Noggin (controls: 11.55 ± 2.33 hr. Noggin: 21.46 ± 4.35hr). This increase does not alter M and G2 phase durations (M: controls: 0.44 ± 0.08hr; Noggin: 0.45 ± 0.13hr, p=0.89; G2: controls: 0.87 ± 0.81hr; Noggin: 1.04 ± 0.21hr, p=0.099). Instead, the increased cell cycle length comes from increased G1 (controls: 7.33 ±1.39hr; Noggin: 14.11 ± 1.51hr, p=0.003) and S durations (controls: 2.93 ± .67hr; Noggin: 5.85 ± 2.5hr, p=0.012). We note that the G2-M transition occurs at the apical surface in lateral neural plate, but sub-apically at the MHP. Interestingly, a subset of Noggin-electroporated cells also undergo G2-M transition sub-apically, recapitulating the MHP phenotype. These results suggest that BMP blockade reduces the apical surface at the MHP by preventing nuclei from becoming apical and mitotic. This is achieved by increasing G1 and S durations and by a sub-apical G2-M transition. These results suggest that BMP signaling regulates MHP formation by modulating both apical junctions and cell cycle kinetics.

Disclosures: C.Y. Brown: None. **D. Eom:** None. **S. Agarwala:** None.

Poster

220. **Patterning of Forebrain**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 220.01/A12

Topic: A.01. Brain Patterning

Support: : NIH R01 DA0018826

Title: Mice with reduced Met signaling in cortex show altered cortical lamination

Authors: *J. M. SMITH, E. M. POWELL;
Anat. and Neurobio., Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Met, the receptor for hepatocyte growth factor/scatter factor (HGF/SF), has been implicated in a number of cellular processes, including proliferation, migration, survival, and process formation. MET has also been identified as a susceptibility locus for autism spectrum disorders (ASD). Both HGF/SF and Met are known to be expressed in the cerebral cortex during development, and Met signaling has previously been implicated in the development of the telencephalon. Alterations in cortical development and connectivity are thought to be involved in the etiology of neurodevelopmental disorders such as ASD.
Our lab has previously shown that mice expressing a kinase-dead Met in the cerebral cortex show altered cortical morphology, including regionally increased cortical volume and surface area, as well as increased thickness of the corpus callosum. We thus sought to determine if cortical lamination was altered in these mice, and which cell populations might be affected. We found that loss of Met signaling in the cortex had altered superficial layer morphology, while deep layers were unaffected. We confirmed this by examining the distribution of several layer-specific markers in these mice. In addition to changes in superficial layer thickness, we also found that cells expressing superficial markers were abnormally distributed in Met-deficient cortex. Notably, we did not find any alteration in the number of callosal projection neurons in Met mutant mice, suggesting that the change in callosal thickness in these mice is not due to an increase in the number of these neurons.

Disclosures: J.M. Smith: None. **E.M. Powell:** None.

Poster

220. **Patterning of Forebrain**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 220.02/A13

Topic: A.01. Brain Patterning

Support: NIH grant 5R01MH081187

Title: A forward genetic screen in mice identifies mutants with abnormal cortical patterning

Authors: *S. HA[1,2], R. W. STOTTMANN[3,2], D. R. BEIER[1,2];
[1]Developmental Biol. and Regenerative Med., Seattle Children's Res. Inst., Seattle, WA; [2]Genet. Div., Brigham and Women's Hosp., Boston, MA; [3]Divisions of Human Genet. and Developmental Biol., Cincinnati Children’s Hosp. Med. Ctr., Cincinnati, OH

Abstract: Formation of a six-layered cortical plate and axon tract patterning are key features of cerebral cortex development. Abnormalities of these processes may be the underlying cause for a range of functional disabilities seen in human neurodevelopmental disorders. To identify mouse mutants with defects in cortical lamination or corticofugal axon guidance, *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis was performed using mice expressing *LacZ* reporter genes in layers 2/3 and 5 of the cortex (*Rgs4-lacZ*) or in corticofugal axons (*TAG1-tau-lacZ*). Four lines with abnormal cortical lamination have been identified. Positional cloning revealed one of these to be a splice site mutation in *Reelin* (*Reln*) that results in a premature stop codon and the truncation of the C-terminal region domain of reelin. Interestingly, this novel allele of *Reln* did not display cerebellar malformation or ataxia, and this is the first report of a *Reln* mutant without a cerebellar defect. Three lines with abnormal corticofugal axon development were also identified, one of which was found by whole-genome resequencing to carry a mutation in *Lrp2*. These findings demonstrated that the application of ENU mutagenesis to mice carrying transgenic reporters marking cortical anatomy is a sensitive and specific method to identify mutations that disrupt patterning of the developing brain.

Disclosures: S. Ha: None. **R.W. Stottmann:** None. **D.R. Beier:** None.

Poster

220. **Patterning of Forebrain**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 220.03/A14

Topic: A.01. Brain Patterning

Support: NIH Grant NS-064587

NIGMS Grant P50-GM076516

Title: Disruption of BMP-FGF mutual inhibition leads to developmental delay and abnormal border formation in the developing telencephalon

Authors: E. S. FUNG[1], L. T. DOAN[2], J. S. HU[1], D. T. TRUONG[1], H. H. JUN[1], R. M. PHAM[1], K. POURHOSSEINI[1], *E. S. MONUKI[2];
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Abstract: Patterning and specification of embryos and tissues is governed by morphogens. In many developmental systems, morphogens generate signaling gradients that are converted into switch-like (“ultrasensitive”) cellular responses, which produce discrete cell types separated by sharp borders. In the dorsal telencephalon, mutually inhibitory bone morphogenetic protein

(BMP) and fibroblast growth factor (FGF) signaling gradients interact within cells to generate ultrasensitive BMP target responses implicated in cell fate and border specification (Hu et al., PNAS 2008; Srinivasan et al., submitted). Presence of FGFs at sub-threshold BMP concentrations suppresses expression of BMP target genes but has little effect at maximal BMP concentrations. Addition of translation inhibitor cycloheximide abolishes this suppression suggesting FGF mediated effects are translation dependent (Fung et al., SFN abstract 2012). The question that remains is how FGFs are involved in the formation of a sharp, robust cortex-dorsal midline border. Here we further define the role of FGF signaling in this ultrasensitivity phenomenon. We hypothesize that disruption of FGF signaling will abolish ultrasensitivite BMP target responses leading to reduced refinement of cortex - dorsal midline border. Preliminary data suggests that FGF signaling mediates slow suppression of BMP target genes leading to refinement of cortex - dorsal midline border in the developing telencephalon. Disruption of FGF signaling leads to a failure of border refinement and developmental delay with possible partial catch up. Normal development will be studied in X-gal stained ex vivo explants and coronal sections of embryonic brains of BRE-gal reporter mice (Doan et al., Plos One 2012). FGF signaling will be disrupted using pan FGF receptor inhibitor PD173074 and effects will be observed in both ex vivo and in vivo. Our findings suggest that this mutual inhibitory signaling is crucial to sharp border formation in the developing telencephalon and may be a common mechanism in various biological systems.

Disclosures: E.S. Fung: None. **E.S. Monuki:** None. **L.T. Doan:** None. **J.S. Hu:** None. **D.T. Truong:** None. **H.H. Jun:** None. **R.M. Pham:** None. **K. Pourhosseini:** None.

Poster

220. **Patterning of Forebrain**

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Topic: A.01. Brain Patterning

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ERC GRANT 309 633

Title: Sharp borders of gene expression in germinal layers identify a protomap of cerebral cortex folding

Authors: *C. D. ROMERO[1], C. BRUDER[2], V. BORRELL[1];
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Abstract: The cerebral cortex is a modular structure organized in distinct cytoarchitectonic and functional areas, limited by sharp borders and distributed along the rostro-caudal and latero-medial axes. In mammals with a folded cortex this modularity also requires the three-dimensional accommodation of the neuronal layers in order to form convolutions along the cortical mantle. All these different modules of cortical neurons appear during development from embryonic neural stem (eNSCs) and progenitor cells arranged in proliferative layers. We recently demonstrated that in several gyrencephalic species, including humans, the differential expansion of cortical domains correlates with sharp differences in eNSC proliferation between adjacent regions in germinal layers. Given the tight genetic regulation of the cell cycle and proliferation of germinal cells along cortical development, we proposed that proliferation differences between prospective folds and fissures might result from differences in expression of certain genes among cortical germinal layers during embryonic development. Here we performed a gene expression analysis of germinal layers in the developing cerebral cortex of the gyrencephalic ferret to characterize differences between adjacent cortical areas with different degrees of expansion/folding. We have found thousands of differentially-expressed genes that defined unique transcriptional fingerprints for each cortical area and germinal zone analyzed. We found the existence of mosaics of gene expression along each of the germinal layers correlating with gyrus/sulcus formation. Significantly, expression patterns were frequently characterized by sharp, step-wise changes in expression levels for genes which are otherwise present shallow gradients across the mouse cortex. Our findings are consistent with the existence of a transcriptional protomap of cortical folding in germinal layers. Differentially-expressed genes between gyrus and sulcus had a 7-fold bias towards genes mutated in human cortical malformations, including lissencephaly, microcephaly and polymicrogyria. This confirms the validity of our strategy to identify genes critically implicated in regulating cortical folding, and hence indicates that others of our differentially-expressed genes may be involved in patterning cortical folding. Furthermore, these findings also indicate that our list of genes differentially expressed is a valid entry point to search for genes mutated in human cortical malformations but for which specific genetic mutations have not yet been identified.

Disclosures: C.D. Romero: None. **V. Borrell:** None. **C. Bruder:** None.

Poster

220. **Patterning of Forebrain**

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Topic: A.01. Brain Patterning

Support: Department of Biotechnology, Govt. of India (ST)

Title: Distinct critical periods for medio-lateral fate specification in the cortical primordium

Authors: *A. S. SHETTY[1], G. GODBOLE[1], E. MONUKI[2], S. TOLE[1];
[1]TIFR, Mumbai, India; [2]Univ. of California, Irvine, CA

Abstract: The cortical primordium requires LIM-HD transcrtiption factor Lhx2 for its specification. In the Lhx2 null mutant, two non cortical structures, the hem and the antihem, expand at the expense of the cortical primordium. This expansion is known to have a critical period that ends at E10.5, after which loss of Lhx2 does not cause expansion of the hem and the antihem (Mangale et al., 2008). Since the hem and the antihem normally form some distance from each other, at the medial and lateral extremes of the dorsal pallium respectively, we hypothesized that they may become specified at different times. We used a conditional Lhx2 knockout line and tamoxifen-inducible CreER to generate a precise temporal map of Lhx2 requirement in the E8.5-E10.5 window. We find that the critical period for hem specification ends significantly earlier than E10.5. Loss of Lhx2 in the E9.5-E10.0 window spares some medial cortex and causes a smaller expansion of the hem. In contrast, the antihem expansion is similar to that in the null mutant. Ongoing experiments are aimed at examining the consequences of these temporal differences in the role of Lhx2 in the earliest stages of cortical development.

Disclosures: A.S. Shetty: None. **G. Godbole:** None. **E. Monuki:** None. **S. Tole:** None.

Poster

220. **Patterning of Forebrain**

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Topic: A.01. Brain Patterning

Support: CAPES - PROEX

FAPESP

CNPq

FAEPA

Title: Immunohistochemical characterization of a cortical neuronal type expressing a combination of markers in the adult rat brain suggesting cell immaturity

Authors: *A. FERNANDES, N. GARCIA-CAIRASCO;
Physiol., Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract: Cortical dysplasia is the most common cause of surgical treatment of drug-resistant seizures in children and it has been widely studied since described in the early 70's. In order to help understanding the mechanisms involved in the formation and pathophysiology of dysplastic lesions, scientists use animal models of disruption of the cortical structure including, as proposed by Dvorák and Feit (Acta Neuropathol. 15;38(3):203-12, 1977), a freezing insult to the developing brain cortex in newly born rodents causing the formation of a microgyrus.
Using the above mentioned method, we induced the cryogenic lesion in ice cold anesthetized neonatal rats (Ethics Committee approval 133/2009) and observed the alterations up to 6 months later. Intriguingly, besides the microgyrus formation, we observed the presence of an unusual neuronal type in the neocortex. We called it Abnormal Pyramidals (AP), due to their densely stained small pyramidal shape with Neu-N immunohistochemistry. These cells are normally organized with their apical processes towards the pial surface but it is not rare to find misoriented cells.
We used an immunohistochemical approach to study the cortical alterations caused by the cryolesion so the APs were studied using the following techniques: neuronal (Neu-N), glial (GFAP), Layer V pyramidal neurons (ER81), Layers III and VI pyramidal neurons (Tbr1) and GABAergic interneuron (Parvalbumin) markers. We found that regions presenting the microgyric lesion were absent of APs.
The APs are present in both experimental and control subjects of the two temporal windows (72 and 180 days) what suggests it is an unusual but stable alteration of the cortical anatomy. This was found in 56% (9/16) of controls and 60% (16/27) of experimental animals with no differences independently of time window or gender (Fisher exact test; $p>0.05$). Those cells were found exclusively in the neocortex, usually in the superficial layers of motor and somatosensory areas.
The analyses of the immunostaining markers showed that APs are stained for all the markers except Parvalbumin, that absolutely lacks in regions affected by the APs.
We first suspected of the anesthesia by cold as a possible culprit for the APs emergence but in a new group of 8 animals experiencing only maternal separation for 3 hours (as the experimental/sham groups) and no exposure to cold we found the same proportions of APs appearance. It is yet inconclusive the nature of those cells, however it is tempting to assume that due to the expression of the various markers but PV those cells could be, actually, immature neurons. Further experiments, coupled to electrophysiology, are needed to elucidate this question.

Disclosures: A. Fernandes: None. **N. Garcia-Cairasco:** None.

Poster

220. **Patterning of Forebrain**

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Topic: A.01. Brain Patterning

Support: NIH Grant R37 AG024102

Title: Longitudinal change in cortical thickness: Freesurfer cross sectional and longitudinal processing streams

Authors: *S. L. WILLIS[1], P. RAST[2], D. MCLAREN[3], K. W. SCHAIE[1], T. J. GRABOWSKI[4]; [1]Seattle Longitudinal Study. Univ. of Washington, SEATTLE, WA; [2]Psychology, Univ. of Victoria, Victoria, BC; [3]Radiology, Massachusetts Gen. Hosp., Charlestown, MA; [4]Neurology, Radiology, Univ. of Washington, Seattle, WA

Abstract: It has been suggested that Freesurfer's longitudinal processing stream produces more accurate and significant results, yet an empirical comparison has yet to be conducted using longitudinal growth models. Here we report on findings on longitudinal change in cortical thickness in a healthy adult sample of N = 163 participants in the Seattle Longitudinal Study (Mage = 62 (age 52 - 87) at time of first scan using two Freesurfer methods. Subjects underwent three scans at two-year intervals (2007, 2009, 2011) on a Philips 3T Achieva scanner. Cortical thickness estimation was performed with FreeSurfer version 5.1 (http://surfer.nmr.mgh.harvard.edu/). The following analysis was conducted separately for the cross-sectional thickness estimations (CS) and the thickness estimates using the longitudinal processing stream (LP). Cortical thickness values for each of the 68 parcels defined by the Desikan parcellation (Desikan et al., 2006) were extracted by subject and timepoint. Multivariate multilevel models were applied using a stepwise modeling procedure to examine longitudinal change across 3 timepoints focusing on association cortices. The first step involved examination of changes in cortical thickness in frontal, parietal, temporal and occipital areas. We started with a baseline model, followed by introduction of explanatory variables (APOE genotype, hypertension) to obtain full models used to explain individual differences in rate of cortical thinning. LP models yielded a greater number of significant main and interaction effects. Findings indicated significant time main effects for the parietal and occipital regions, with a significant time effect for the temporal region only with LP. A Time x APOE interaction was found for CS and LP for the frontal region, but only for LP for the parietal and temporal regions.

A Time x AgeT1 was found for the frontal region for LP, but not CS; both analyses indicated a Time x AgeT1 for the temporal region. These results provide some evidence that the LP improves the significance even for more advanced modeling approaches through reduced within subject variance

Disclosures: S.L. Willis: None. **P. Rast:** None. **D. McLaren:** None. **K.W. Schaie:** None. **T.J. Grabowski:** None.

Poster

220. **Patterning of Forebrain**

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Topic: A.01. Brain Patterning

Support: KAKENHI

GCOE

Title: Inner fiber layer-like structures revealed with *In utero* electroporation in ferrets

Authors: *H. KAWASAKI[1,2], T. TODA[1,2], L. IWAI[2], K. TANNO[2];
[1]Sch. of Med, Kanazawa Univ., Ishikawa, Japan; [2]The Univ. of Tokyo, Grad. Sch. of Med., Tokyo, Japan

Abstract: Brain structures such as the inner fiber layer (IFL) and the outer subventricular zone (OSVZ) in the developing cerebral cortex are especially prominent in higher mammals such as primates. However, the mechanisms underlying the formation of these structures in the cerebral cortex of higher mammals are not completely understood, mainly because genetic manipulations that can be applied to the cerebral cortex of higher mammals was poorly available. At Neuroscience 2012 last year, we reported a rapid and efficient genetic manipulation technique for the cerebral cortex of gyrencephalic carnivore ferrets using in utero electroporation (Kawasaki et al., 297.01). Here we show that transgenes can be expressed most of cortical layers and germinal zones using in utero electroporation in ferrets. The morphology of GFP-positive cells in the OSVZ was clearly visible even without immunostaining, and multiple genes were efficiently co-expressed in the same cells. We also uncovered that fibers which seemed to correspond to those in the IFL of monkeys also existed in ferrets and were derived from newly generated cortical neurons. These results suggest that the IFL is conserved in primates and carnivores, and that it should be useful to combine ferrets and in utero electroporation for

investigating the mechanisms underlying the formation of the cerebral cortex in higher mammals.

Disclosures: H. Kawasaki: None. **T. Toda:** None. **L. Iwai:** None. **K. Tanno:** None.

Poster

220. **Patterning of Forebrain**

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Topic: A.01. Brain Patterning

Support: NIMH 5R03MH080502-02

Title: Bilateral Enucleation at birth alters intraneocortical connections in early postnatal development

Authors: *O. O. KOZANIAN, K. J. HUFFMAN;
Dept. of Psychology, UC Riverside, Riverside, CA

Abstract: Functional sensory and motor areas in the developing mammalian neocortex are generated through a complex interaction of cortically intrinsic mechanisms, such as gene expression, and cortically extrinsic mechanism such as those mediated by thalamic input. There is supporting evidence for the involvement of both mechanisms in cortical patterning and the establishment of areal boundaries in early development. However, the nature of the interaction between intrinsic and extrinsic processes is not well understood. We have previously used a perinatal bilateral enucleation mouse model to test some aspects of this interaction by reweighting sensory input to the developing cortex and examining gene expression, a read–out of a potential intrinsic mechanism. Ten days after bilateral enucleation, cortical gene expression and developing sensory intraneocortical connections (INCs) were examined. Visual deprivation at birth resulted in a shift of INCs at the border between somatosensory and visual cortex (S-V). These aberrant connections co-registered with ectopic *Ephrin A5* expression in the same location in enucleated mice (Dye et al., 2012). In the present study, we extend this research by investigating the development of the phenotype prior to post-natal day (P) 10. Somatosensory and visual INCs were indistinguishable between P1 mice bilaterally enucleated at birth and P1 controls. By P4, a medial shift of retrogradely labeled cells resulting from a somatosensory cortex dye placement was observed in enucleated mice. These ectopic cells were present in an area that would be designated visual cortex in a normal, sighted mouse. Thus, the phenotype we observed previously in the P10 mouse bilaterally enucleated at birth begins to form as early as P4, just 4 days after bilateral enucleation, and 8 days prior to eye opening.

Disclosures: O.O. Kozanian: None. **K.J. Huffman:** None.

Poster

220. **Patterning of Forebrain**

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Topic: A.01. Brain Patterning

Support: NICHD 4R00HD058044

Title: The development of the corpus callosum is dependent on FGF8 signaling

Authors: *K. M. CORELLA[1], W. C. J. CHUNG[1,2];
[1]Sch. of Biomed. Sci., [2]Biol. Sci., Kent State Univ., Kent, OH

Abstract: Agenesis of the corpus callosum (ACC) is one of the more common clinical brain malformations found in patients with mental retardation, seizures, visual problems, and in more severe cases, holoprosencephaly. Yet, there is still little known about the underlying cause of ACC in these patients. Here, we explored whether fibroblast growth factor (FGF) signaling plays a role in the development of the corpus callosum. Specifically, FGF 8 has been shown to be especially important for normal embryonic neocortical development. For instance, *Fgf8* hypomorphic mice have severe morphological defects in developing neocortical-related structures, including an absence of the corpus callosum and cortical thinning. Since FGF8 has previously been implicated in axonal guidance, we hypothesized that the failure of corpus callosum formation is due to abnormal local FGF8 signaling through its cognate receptor FGF receptor (FGFR) 1. This defect in FGF8/FGFR1 signaling may have disrupted midline guidance cues. Using in situ hybridization, we showed that *Fgfr1* mRNA expression was reduced in the indusium griseum of homozygous *Fgf8* hypomorphic mice at postnatal day (PN) 0. In a second study, we found that glial fibrillary acidic protein-expressing astrocytes that form the glial midline zipper region were eliminated in PN 0 homozygous *Fgf8* hypomorphic mice as compared to their wildtype littermates. Furthermore, we have preliminary evidence indicating that corpus callosum dysgenesis may, in part, be the consequence of the abnormal neocortical organization found in *Fgf8* hypomorphic mice. Indeed, *Dickkopf* (*Dkk*) 3 mRNA expression, a marker specific for cortical layers IV/V, was reduced in homozygous *Fgf8* hypomorphic mice. In contrast, *FERM domain containing* 4b (*Frmd4b*) mRNA expression, a marker specific for cortical layers I/II, seemed to be unaffected by the deficiency in FGF8 signaling. Together, we infer that ACC in mice may be related to the FGF8-dependent loss of *Dkk3* expression in the cortical layers IV/V, in which a subset of callosal projecting neurons reside.

Disclosures: K.M. Corella: None. **W.C.J. Chung:** None.

Poster

220. **Patterning of Forebrain**

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Support: Research Corporation for Science Advancement 19482

CWU Science Honors Program

CWU STEP program

Title: Identification of proteins that interact with neocortical arealization factors in the developing neocortex

Authors: *T. T. KROLL[1], J. A. GROVES[2], M. J. MULLAN[1], J. BERG[1], C. MILLER[1], A. M. MILLER[1], N. MEYERS[1];
[1]Chem., Central Washington Univ., Ellensburg, WA; [2]Biol. Chem., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: The landscape of the rodent neocortex is dominated by four major areas that process discrete classes of information. The size and position of these areas is regulated by a group of neocortical arealization factors that include Emx2, Sp8, and Pax6. These proteins share three common traits: (1) they are all transcription factors, (2) they are each expressed in a graded fashion within the neocortical progenitor cells, and (3) they are not seen in significant concentrations within the daughter neurons that later compose the mature neocortex, indicating that they acquire their positional information from their respective progenitor cells. When considering the general importance of protein-protein interactions in mediating transcription factor activity, there is a surprising lack of knowledge as to the role that these types of contacts play in regulating neocortical arealization. In order to fill this void, we have initiated a series of screens to identify these protein-protein interactions, beginning with Emx2 and continuing with Sp8 and Pax6. Here, we report our initial results obtained using full-length Emx2 and significant fragments of Sp8 and Pax6 in yeast two-hybrid screens. Two of the proteins retrieved from the Emx2 screen include QkI-7, an RNA-binding protein, and Cnot6l, which belongs to the large CCR4-NotI complex and contains 3'-5' poly(A) exoribonuclease activity. Potential Pax6 binding partners identified in the yeast two-hybrid screen include (poly)ubiquitin C, rRNA promoter binding protein, and ribosomal protein S20-like. Finally, we have identified potential binding

partners for Pax6 that include Celf1, an RNA binding protein involved in mRNA splicing and translation, as well as ribosomal protein S20-like, and eIF5A. Collectively, these screens suggest a potential role for the transcription factors involved in neocortical arealization that extends beyond regulating transcription. We propose that these protein-protein interactions may be regulating the packaging, transport, and translation of transcripts that are directed to the newly generated daughter cells during neocorticogenesis, thus providing these neurons with the positional information necessary to carry out their future functional roles within the neocortex.

Disclosures: T.T. Kroll: None. **J.A. Groves:** None. **M.J. Mullan:** None. **J. Berg:** None. **C. Miller:** None. **A.M. Miller:** None. **N. Meyers:** None.

Poster

220. **Patterning of Forebrain**

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Program#/Poster#: 220.12/A23

Topic: A.01. Brain Patterning

Title: Neuronal patterning in the cerebral cortex of forebrain-specific Ctgf Knockout Mice

Authors: *K.-C. CHEN;
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Abstract: Connective tissue growth factor (CTGF), a member of CCN family, plays important roles in cell adhesion, migration, differentiation and survival. In the cerebral cortex, Ctgf is primarily expressed in subplate neurons. In order to comprehend the roles of Ctgf in cortical development, a forebrain-specific *Ctgf* knockout mouse model was generated by crossing *Ctgf* $^{flox/flox}$ mice with Emx1-*Cre* transgenic mice. *Ctgf* $^{flox/flox}$; Emx1-*Cre* mice were fertile and developed normally. The expression of Ctgf in the subplate was abolished in knockout mice while other subplate markers such as Nurr1 and Complexin 3 were still present, indicating that the identity of subplate neurons was not affected by *Ctgf* deletion. Compared with wildtype and *Ctgf* $^{+/+}$; Emx1-*Cre* mice, no gross abnormality was noticed in the brain and the body of forebrain-specific CTGF knockout mice. In the cerebral cortex, the distribution of NeuN-, Gad67- and Iba1-positive cells were comparable between genotypes, suggesting the migration and survival of cortical neurons and microglia were not affected while Ctgf was devoid in the subplate. Together, our data suggest the cortical neuronal patterning was not determined by Ctgf expression in the subplate.

Disclosures: K. Chen: None.

Poster

220. **Patterning of Forebrain**

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Support: NIH Grant NS054273

NIMH Contract HHSN-271-2008-0047

Title: Transcriptome analysis of postnatal development of rhesus macaque hippocampus and identification of conserved molecular signatures of adult dentate gyrus neurogenesis

Authors: *J. A. MILLER[1], A. BERNARD[1], J. NATHANSON[2], D. FRANJIC[3], S. SHIM[3], R. A. DALLEY[1], S. SHAPOURI[1], K. A. SMITH[1], S. M. SUNKIN[1], J. L. BENNETT[4], C.-K. LEE[1], M. J. HAWRYLYCZ[1], A. R. JONES[1], D. G. AMARAL[4], N. ŠESTAN[3], F. H. GAGE[2], E. S. LEIN[1];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Lab. of Genet., The Salk Inst. for Biol. Studies, La Jolla, CA; [3]Dept. of Neurobio. and Kavli Inst. for Neurosci., Yale Univ. Sch. of Med., New Haven, CT; [4]Dept. of Psychiatry and Behavioral Sci. and The M.I.N.D. Inst., UC Davis, Sacramento, CA

Abstract: Non-human primates provide a useful test bed for studying neurodevelopment, allowing many of the same experimental controls possible with rodent models, while also having brains that diverged from the human lineage much more recently. Here, we present a transcriptome-wide survey of the male rhesus monkey hippocampus in the newborn (0 months), infant (3 months), juvenile (12 months) and young adult (48 months), with major anatomical and cellular partitions independently isolated using laser microdissection and profiled using DNA microarrays. This design allowed us to pursue several parallel avenues of transcriptional analysis using a single data set. We first sought to identify genes showing differential expression patterns between hippocampal compartments. Several thousand genes separated subregions into three groups based on primary cell type--pyramidal, GABAergic and glial, or granule cells--representing by far the largest transcriptional effect. Furthermore, we could identify genes specifically enriched in nearly all compartments. We next looked for genes showing changes in expression patterns across postnatal development. Many such genes showed an increase or decrease in expression between newborn and infancy, consistent with the anatomical observation that portions of the hippocampus continue to develop postnatally. Interestingly, the subgranular zone, which is one of only two brain areas with confirmed neurogenic potential in adult, shows

the most striking changes with time of any hippocampal compartment. As a final analysis, we compared these data to comparable transcriptome-wide assays in mouse to identify common signatures of postnatal neurogenesis. We find that the subgranular zone contains diverse cell types, including progenitor and dividing cells, immature granule cells, astrocytes, oligodendrocytes, and GABAergic interneurons, and that genes expressed in this layer subdivide based on temporal profiles reflecting maturation of glia versus granule neurons. One such neurogenesis-related gene network has decreasing postnatal expression that is highly correlated with the number of proliferating cells in the dentate gyrus of rhesus monkey, and contains many genes showing similar postnatal downregulation in mouse. Together these results present a comprehensive look at the developing rhesus monkey hippocampus, and provide insight into a biological mechanism conserved between species. All data described herein are publicly accessible through the Allen Brain Atlas data portal at www.brain-map.org.

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Poster

220. **Patterning of Forebrain**

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Support: Wellcome Trust Grant JJCRCPR

Title: Foxg1 modulates midline axon crossing in the zebrafish telencephalon

Authors: *F. CHIARA, C. HOUART;
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Abstract: Commissural axons cross the midline and connect the left and the right side of the nervous system. Various molecules have been implicated in this fundamental process. However, it is still poorly understood how these cues interact with each other to facilitate the precise wiring between the two hemispheres. In zebrafish, axons arising from the anterior dorsal telencephalic neurons (ADt) fail to project rostrally into the anterior commissure in the absence of the transcription factor Foxg1. Instead, they remain in the rostral telencephalon. Transplant experiments show that this phenotype is not caused by a Foxg1 cell-autonomous mechanism. Instead, we found changes in the expression of known axon-guidance genes, such as Netrin and

Slits, in Foxg1 morphants. Taken together, these findings suggest a defect in the correct specification of commissural glia. The transcription factor Foxg1 has been widely studied as a critical regulator of cortical neurogenesis and its loss of function leads to dramatic defects in the organisation of the telencephalon. Here, we present an unexplored role for Foxg1 in the specification of the commissural plate.

Disclosures: F. Chiara: None. **C. Houart:** None.

Poster

220. **Patterning of Forebrain**

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Topic: A.01. Brain Patterning

Support: NSC 101-2321-B-001-048

Title: Characterizing the role of LIM homeobox 2 in area patterning

Authors: C.-F. WANG, *S.-J. CHOU;
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Abstract: The neocortex is the largest region of the mammalian cerebral cortex and is responsible for sensory perception, cognition and control of movement. Tangentially, neocortex is organized into areas, which are functionally unique subdivisions with different cytoarchitecture and gene expression profiles. Previous studies of the mechanisms that pattern the neocortex into areas have led to a model in which transcription factors (TFs) expressed in graded fashion in the ventricular zone (VZ) of dorsal telencephalon (dTel) determine the size and position of the primary areas. LIM homeobox 2 (Lhx2), a LIM-homeodomain transcription factor, has been shown to play critical roles in early cortical development. Deletion of Lhx2 at an early stage alters regional fate specification of the dorsal telencephalon, and results in decreased number of cortical progenitors and premature neuronal differentiation. As Lhx2 is expressed in a high caudal to low rostral pattern in the dTel VZ, it is likely Lhx2 is also involved in area patterning. Using conditional gene inactivation as well as in utero electroporation approaches, we attempt to elucidate the role of Lhx2 in area patterning.

Disclosures: C. Wang: None. **S. Chou:** None.

Poster

220. **Patterning of Forebrain**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 220.16/B1

Topic: A.01. Brain Patterning

Support: R01NS31558

R01MH086147

Title: Sensory cortex regulates top-down plasticity in thalamocortical circuits

Authors: *A. B. ZEMBRZYCKI[1], S.-J. CHOU[1], R. ASHERY-PADAN[2], A. STOYKOVA[3], D. D. M. O'LEARY[1];
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Abstract: Primary somatosensory cortex (S1) contains a complete body map that mirrors subcortical maps developed by peripheral sensory input projecting to hindbrain, thalamus, then S1. Peripheral changes during development alter these maps through ‘bottom-up’ plasticity. Unknown is whether S1 size influences map organization and if an altered S1 map feedbacks to affect subcortical maps. We show that cortex-specific deletion of Pax6 reduces S1, resulting in a reduced cortical map and reproducible loss of body representations by exclusion of later-differentiating thalamocortical input. An initially normal thalamic map is re-patterned to match the aberrant cortical map by apoptotic deletion of thalamic neurons that represent body parts whose axons are excluded from S1. Absent body parts are rescued by reducing competitive imbalance between thalamocortical axons within S1 or increasing S1 size. We conclude that S1 size determines resolution and completeness of body maps and drives ‘top-down’ plasticity that re-patterns thalamus to match S1.

Disclosures: A.B. Zembrzycki: None. **S. Chou:** None. **R. Ashery-Padan:** None. **A. Stoykova:** None. **D.D.M. O'Leary:** None.

Poster

220. **Patterning of Forebrain**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 220.17/B2

Topic: A.01. Brain Patterning

Title: miRNA135a levels determines cortical hem and hippocampus size by modulating embryonic Wnt/BMP signaling

Authors: *G. CARONIA-BROWN[1], A. ANDEREGG[1], R. AWATRAMANI[2];
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Abstract: microRNAs (miRs) regulate gene expression in diverse physiological scenarios, but their role in modulating morphogen function has been less studied, particularly in embryonic CNS. Here, we define a new role for miR135a2 - that of influencing the size of the cortical hem, possibly by modulating Wnt/BMP signaling. miR135a2 was identified as robustly expressed in the cortical hem, the hippocampus (Hp) primordium, and choroid plexus, overlapping expression of several Wnts/BMPs. miR135a2 is predicted to target several molecules in the Wnt/BMP pathways. To investigate a potential role for miR135a2 in modulating Wnt/BMP signaling, we conditionally over-expressed miR135a2 in the dorsal forebrain using Emx1-Cre line as a driver. In E12.5 Emx1::Cre;miR135a2OE, we observed a reduction in the size of the hem and the Hp primordium; the choroid plexus was reduced in its complexity and the cortical domain was much smaller than in controls. Overall, early over-expression of miR135a2 phenocopied reduced Wnt/BMP signaling. According to expectations, the smaller hem resulted in a reduction of all Hp structures in Emx1::Cre;miR135a2OE. When Nestin::Cre, which initiates recombination at ~E11.0, was used as a driver, we observed no change in hem or hippocampus size, suggesting that miR135a2 levels determine hem size during an early time window.

Disclosures: G. Caronia-Brown: None. **A. Anderegg:** None. **R. Awatramani:** None.

Poster

220. **Patterning of Forebrain**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 220.18/B3

Topic: A.01. Brain Patterning

Support: Neurodis foundation

LabEX CORTEX

Title: Mapping the gene regulative networks of cortical development

Authors: *U. BORELLO[1], M. MADHAVAN[2], K. CAMPBELL[2], C. DEHAY[1];
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Abstract: The transcriptional network regulating cortical development remains relatively unknown. Molecules secreted by the different forebrain signaling centers establish the expression gradient of specific transcriptional factors, which are necessary for the patterning and differentiation of the distinct structural and functional domains of the cerebral cortex.
To get new insights in the transcriptional regulation of corticogenesis, we have focused on the role of Sp8, a zinc finger transcription factor involved in early patterning and arealization of the cortex.
In order to decipher the molecular mechanisms of Sp8 regulation, we have used a new genetic system to over-express Sp8 specifically in the forebrain at different time points during development. Gain-of-function approach allowed us to examine the role of Sp8 in proliferation, survival and differentiation of cortical progenitors and, importantly, its relationship with Fgf signaling. We have identified a novel regulatory feedback loop at the core of the molecular mechanism controlling patterning and growth of the developing cortex.
Given the fundamental role of Sp8 in patterning the developing cortex and maintaining the balance between neural progenitor cell renewal/differentiation, we are studying the network of genes upstream and downstream of Sp8. We are implementing a genome wide analysis of the Sp8 downstream genes in the gain-of-function as well as loss-of-function Sp8 mouse mutants. In parallel, we are studying the enhancers regulating Sp8 expression during corticogenesis to identify the gene upstream of Sp8.
A comparative analysis of the molecular mechanisms regulating gene expression between mouse and non-human primates will allow us to understand how evolution shaped the primate brain.

Disclosures: U. Borello: None. **M. Madhavan:** None. **K. campbell:** None. **C. Dehay:** None.

Poster

220. **Patterning of Forebrain**

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Program#/Poster#: 220.19/B4

Topic: A.01. Brain Patterning

Support: 5-T32-NS007180-30

R01-NS-56243-05

R01-NS-38690-11

Title: Fate-labeling of Islet1 population shows pervasive progeny in mouse forebrain

Authors: *F. SIDDIQI[1], A. L. TRAKIMAS[2], T. T. CLARKE[1], E. D. MARSH[1,3], J. H. WOLFE[1,3];
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Abstract: In developing mammalian ganglionic eminence (GE), unique progenitor domains exist that are defined by their respective transcription factor expression patterns known to generate interneuron subtypes. Specifically, the LIM homeodomain gene, Islet1, is expressed in the ventral division of the LGE and is known to generate striatal neurons. It remains unknown if Islet1 derived progeny migrate beyond the basal ganglia and inhabit other forebrain structures, indicating a larger unidentified role of Islet1 in brain development. In the current study, we sought to determine the complete lineage generated by Islet1 progenitor pool. Immunostaining showed that Isl1 is expressed at embryonic day (E) 12.5 in developing thalamus and ganglionic eminence and is down-regulated postnatally. We thus utilized a Cre/loxP mediated knock-in mouse line whereby the Islet1 gene locus drove expression of Cre recombinase and was crossed with floxed R26-stop-eYFP mutant mice to permanently label Islet1-derived cells following recombination. At postnatal day (P) 0, eYFP+ cells derived were found in multiple forebrain structures. Cells were located in olfactory bulb, hippocampus, thalamus and cerebral cortex, in addition to striatum. Based upon morphology, these cells were immature neurons. In neocortex, eYFP+ neurons were both radially and tangentially aligned and spanned multiple cortical layers. At P21, eYFP+ cells persisted into adulthood in the above mentioned structures indicating a postnatal function of Islet1-derived progeny. Immunohistochemistry showed some of these cells were glia. Overall, these findings suggest Islet1+ progenitors regulate production, differentiation, and migration of multiple neuronal and glial subtypes.

Disclosures: F. Siddiqi: None. **A.L. Trakimas:** None. **T.T. Clarke:** None. **E.D. Marsh:** None. **J.H. Wolfe:** None.

Poster

220. **Patterning of Forebrain**

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Topic: A.01. Brain Patterning

Support: NIH Grant R01 NS31558

NIH Grant R01 MH086147

NIH Fellowship T32 EY020503

Title: Emx1 influences area patterning in a background dependent fashion

Authors: *A. M. STOCKER[1], S. SAHARA[1], A.-L. S. DONAHOO[2], L. J. RICHARDS[2], D. D. M. O'LEARY[1];
[1]Mol. Neurosci., Salk Inst., LA JOLLA, CA; [2]Queensland Brain Inst., The Univ. of Queensland, Brisbane, Australia

Abstract: Establishing appropriate area patterning in the neocortex is a critical developmental event, and several graded transcription factors expressed by cortical progenitors have been implicated in this process, including Emx2. Despite the close homology to Emx2, both in sequence and cortical expression, Emx1 has not been implicated in area patterning. Emx1 is expressed in a low anterior-lateral to high posterior-medial gradient across the embryonic cortex, in both progenitors and neurons. To determine if Emx1 does influence area patterning, we examined two different KO lines on different genetic backgrounds. A constitutive Emx1 deletion on a C57BL/6 background that had been backcrossed for 9 generations did not exhibit any area patterning phenotype. However when the backcrossed C57BL/6 Emx1 KOs were crossed with 129S mice to generate a mixed genetic background, we observed an areal phenotype reminiscent of the Emx2 heterozygous KO. Specifically, we find that in these KOs anterior areas (frontal/motor) increase in relative size, posterior areas (visual and auditory) decrease in size, and overall, areas shift posteriorly. We also observed changes in area patterning in the second Emx1 KO line, where Cre-recombinase has been knocked into the Emx1 locus on the C57BL/6 background (Emx1 KI). However these animals exhibit a large decrease in overall cortical size, making it difficult to determine changes specific to area patterning. To circumvent these issues we crossed the Emx1 KI and Emx1 KOs, with the mixed genetic background, to generate another Emx1 KO (Emx1 KI/KO). An overall posterior shift in areas was also observed in these new Emx1 KI/KOs, where posterior areas decreased in size and anterior areas increased in size. The areal phenotype of the Emx1 KI/KOs was similar to that seen in KIs without the dramatic reduction in cortical size, and more pronounced than that of the KOs on a mixed background. In conclusion, we show that Emx1 does play a significant role in specifying area identities.

Disclosures: A.M. Stocker: None. **D.D.M. O'Leary:** None. **A.S. Donahoo:** None. **L.J. Richards:** None. **S. Sahara:** None.

Poster

220. **Patterning of Forebrain**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 220.21/B6

Topic: A.01. Brain Patterning

Support: NIH grant R01 MH067715 (F.M.V.)

Title: FGF2-induced gyrification of the mouse cerebral cortex suggests that the emergence of gyri and sulci is molecularly encoded in the primordial cortical wall

Authors: *S. TOMASI[1], B. G. RASH[1], H. D. LIM[1], C. SUH[1], F. M. VACCARINO[1,2,3];
[1]Child Study Ctr., [2]Dept. of Neurobio., [3]Kavli Inst. for Neurosci., Yale Univ., New Haven, CT

Abstract: The cerebral cortex is made of modular units, the cortical columns, whose number in each cortical area is thought to affect processing power and therefore specific cognitive abilities. The number of cortical columns affects cortical surface area, and hence the folding of the cortical mantle, which varies considerably across species and evolution. Gyrification is thought to represent an optimal evolutionary strategy to increase cortical area considerably, allowing for greater functionality without increasing cranial size. Fibroblast Growth Factors (FGFs) were previously shown to play a critical role during brain development by regulating the rate of proliferation of progenitor cells and their differentiation into mature neurons, therefore we investigated whether excess FGF signaling would affect the trajectory of neurogenesis and induce an overall change in brain size and shape. Injections of FGF2 protein into E11.5 brain primordia (prior to the onset of neurogenesis and the formation of axonal connections) induced the formation of prominent bilateral gyri and sulci in the rostrolateral cortex, a phenotype not induced by FGF8b, another FGFR ligand involved in brain patterning. Corresponding to FGF2-induced gyrification was increased tangential growth of the rostral ventricular zone (VZ) as well as increased proliferation of Tbr2+ intermediate neuronal precursors in the subventricular zone (SVZ), with ectopic Er81 expression and elevated Tbr1+ neurogenesis. However, preliminary analyses by means of both single BrdU injections and combined CldU-IdU administration did not reveal substantial alterations in cell cycle length and cell cycle exit in response to FGF2 in both VZ and SVZ of the gyrus-forming region, suggesting that other processes, such as cell fate or cell death, may be involved. .In addition to rostral expansion, FGF2 induced shrinkage of the caudal cortical primordium, including the hippocampus, by disrupting Wnt signaling as revealed by decreased Wnt3a and Lef1 expression in the cortical hem and dentate gyrus, respectively. To elucidate the functional role of single FGF receptors in the gyrification of the cortex, we carried out FGF2 injections in FGFR3+/- mutants and preliminary analysis revealed that some of the FGF2 effects in the enlargement of anterior brain could be at least partially antagonized. To understand the molecular underpinnings of cortical gyrification, our current analysis includes gene expression profile analysis comparing control and FGF2-injected brains using RNA-sequencing. Our data suggest that the location for emergence of cortical gyri and sulci is molecularly encoded in the primordial cortical wall.

Disclosures: S. Tomasi: None. **B.G. Rash:** None. **H.D. Lim:** None. **C. Suh:** None. **F.M. Vaccarino:** None.

Poster

220. **Patterning of Forebrain**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 220.22/B7

Topic: A.01. Brain Patterning

Support: Sloan-Swarz Foundation Fellowship

Title: A metric for non-uniform expansion of cortical sheet during neural development

Authors: *D. D. FERRANTE, Y. WEI, A. KOULAKOV;
Cold Spring Harbor Labs., Cold Spring Harbor, NY

Abstract: Cortical sheet undergoes substantial expansion of its area during neural development. It is not clear how non-uniform this expansion is across the 2D surface of cortex and how these potential non-uniformities are introduced. To investigate the non-uniformity of cortical expansion, we developed a method for isometric embedding of cortical sheet into the 3D space. Isometric embedding in differential geometry preserves in-plane metric at every point of embedded manifold. Isometric embedding leads to the unfolding of cortical sheet by introducing bending deformations only without substantial stretching. We show that curved geometry of cortical sheet could not have been generated without substantial differential neurogenesis. To quantify such a non-uniform expansion we propose a model for cortical neurogenesis that allows to predict differentials in neurogenesis across cortical sheet as a function of time.

Disclosures: D.D. Ferrante: None. **Y. Wei:** None. **A. Koulakov:** None.

Poster

220. **Patterning of Forebrain**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 220.23/B8-DP1

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

Support: R01 MH077694

Title: Mapping cortical connectivity during early postnatal development

Authors: *L. A. COCAS, S. J. PLEASURE;
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Abstract: Unraveling the details of mammalian neuronal circuit formation is fundamental in order to understand how the development of neuronal connectivity is affected in developmental disorders such as autism and schizophrenia. Mapping mammalian neural circuitry in vivo has been a challenging biological question that, until recently, was exceedingly difficult to address with existing research tools. A recent technique developed in the Callaway lab using an attenuated rabies virus that spreads monosynaptically and retrogradely to connected neurons has made it possible to analyze presynaptic monosynaptic connections in vivo (Wickersham, et al., 2007). This strategy did not allow for analysis of cell-type specific connectivity, and required two viral injections, making analysis of the earliest developing circuits challenging. We have combined this circuit tracing strategy with genetically labeled mice using a Cre/Tet approach to target different neuronal subtypes during early postnatal cortical development. This approach allows us to analyze the presynaptic connectivity of a variety of different neuronal types using Cre-based cell subtype targeting. It also allows us to control the timing of viral protein expression in vivo as well as timing of infection by driving expression of the viral proteins TVA and G that are necessary, respectively, for infection and monosynaptic spread, with a Tet responsive Cre- dependent reporter. We are able to determine the presynaptic connectivity of excitatory and inhibitory interneurons during early postnatal cortical development. In our hands this approach is a powerful tool to map developing cortical circuits in vivo.

Disclosures: L.A. Cocas: None. **S.J. Pleasure:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 221.01/B9

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

Support: North-Rhine-Westphalia Repatriation Program

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SFB1080

Title: Molecular mechanisms regulating neuronal migration during the development of the dopaminergic system

Authors: *G. O. BODEA[1], J.-H. SPILLE[2], P. ABE[3], A. SENTURK ANDERSSON[4], A. ACKER-PALMER[4], R. STUMM[3], U. KUBITSCHECK[2], S. BLAESS[1];
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Abstract: Midbrain dopaminergic neurons (MbDN) are located in the ventral tegmental area (VTA) and substantia nigra (SN) and are involved in various brain functions including motor control and reward-associated behavior. During embryogenesis, MbDN are generated in a progenitor domain in the ventral midbrain from where they have to migrate and assemble into the laterally positioned SN and the medially located VTA. The migratory behavior of MbDN and the mechanisms regulating MbDN migration are not well understood. Using genetic inducible fate mapping, we can heritably mark two distinct MbDN progenitor populations, which preferentially give rise to either MbDN in the SN or the medial VTA. Analysis of the temporal-spatial changes in the distribution and morphology of these two MbDN subpopulations as well as time-lapse imaging of migrating MbDN in organotypic slice cultures reveals two distinct modes of migration: MbDN destined for the SN migrate first radially from their progenitor domain to the forming mantle layer and subsequently switch to tangential migration to reach their final position in the lateral midbrain. In contrast, neurons destined for the medial VTA only undergo radial migration. We further show that the components of the Reelin signaling pathway are specifically expressed in a lateral MbDN subpopulation during embryonic development, while chemokine receptors are expressed in medially-located MbDN. Using time-lapse imaging and analysis of mouse mutants we demonstrate that Reelin signaling regulates the speed and trajectory of tangentially migrating MbDN and the formation of the SN, while chemokine signaling modulates the radial migration of MbDN. In this study we characterize in detail the distinct migratory pathways taken by MbDN destined for the SN or the medial VTA and we provide insight into the mechanisms that control different modes of MbDN migration.

Disclosures: G.O. Bodea: None. **J. Spille:** None. **P. Abe:** None. **A. Senturk Andersson:** None. **A. Acker-Palmer:** None. **R. Stumm:** None. **U. Kubitscheck:** None. **S. Blaess:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

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Program#/Poster#: 221.02/B10

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

Support: NIH Grant HD63071

Title: Sleep-dependent neural activity in the medial septum as hippocampal theta emerges in newborn rats

Authors: *D. MUKHERJEE, M. S. BLUMBERG;
Psychology, The Univ. of Iowa, Iowa City, IA

Abstract: Sensory feedback from self-generated twitches during active sleep triggers neural activity in the hippocampus of newborn rats. Another key feature of active sleep is the firing of hippocampal neurons at theta frequency (4-14 Hz). Direct inputs from the medial septum generate low-frequency theta during quiet wakefulness and tonic active sleep. In contrast, the medial septum mediates high-frequency theta via entorhinal cortex during phasic active sleep (when twitching occurs) and active wakefulness. Mohns and Blumberg (2008, 2010) showed that hippocampal unit activity is tightly coupled to peripheral twitching during active sleep before the emergence of theta at postnatal day (P) 8. Moreover, when theta emerged at P8, it occurred in short bouts that were also tightly coupled to twitching. Given that septo-hippocampal pathways are well established at birth, we hypothesized that sensory feedback from twitches is conveyed to the medial septum, which then exhibits twitch-dependent activity before and after the emergence of theta. After P8, we hypothesize that septal activity generates theta directly, or perhaps indirectly via effects on entorhinal cortex. To test these hypotheses, we recorded extracellular unit activity in the medial septum in unanesthetized rats from P3 to P10. Pups were head-fixed and recordings were performed as they cycled normally between sleep and wakefulness. As predicted, preliminary data in P3-7 rats reveal the presence of sleep-dependent activity of the medial septum in newborn rats, with some units exhibiting increased activity within 200 ms of a twitch. These findings suggest a role for sleep-related twitches in the activity-dependent development of the septo-hippocampal system, which plays a pivotal role in learning and memory in later life.

Disclosures: D. Mukherjee: None. **M.S. Blumberg:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

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Program#/Poster#: 221.03/B11

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

Support: NIH Grant MH091451

NIH Grant DC009910

NIH Grant DC03906

Title: Developmental and maternal regulation of infant brain state

Authors: E. C. SARRO[1,2], R. M. SULLIVAN[1,2], *D. A. WILSON[1,2];
[1]Child and Adolescent Psychiatry, NYU Sch. of Med., New York, NY; [2]Emotional Brain Inst., Nathan Kline Inst. for Psychiatric Res., Orangeburg, NY

Abstract: The survival of the infant is highly dependent on identifying, learning and approaching the maternal caregiver. This process results in the formation of an attachment to the caregiver. However, while it is clear that the maternal caregiver can exert long lasting changes to brain function and behavior, it is not known how neural activity in the infant is directly influenced by interactions with the caregiver or littermates in their natural environment. Here we addressed this by implanting infant rat pups (PN12) with bipolar stainless steel electrodes, targeting the amygdaloid complex and connected to a wireless transmitter (DSI) implanted subcutaneously. We recorded spontaneous local field potential (LFP) activity daily for 7 consecutive days in freely behaving infants. This period includes several important developmental milestones relative to attachment and maturation. Recordings were taken while infants were in their natural environment: with mother and litter in the nest as well while alone, without contact with the mother or litter. Fast Fourier Transform analysis (2.4 Hz bins) was used to quantify LFP oscillatory power in theta (5-15Hz), beta (15-35Hz) and gamma (35-60Hz) bands. Using the alone recording sessions, we were able to make within animal comparisons and describe generalized changes to LFP oscillatory power as animals progress through this period of early development. Moreover, using the daily recording sessions that included mother and littermates, we were able to assess changes in LFP activity during interactions with the mother and littermates, as well during periods of specific behavioral states (i.e., nursing, grooming, milk ejections). We found persistent changes to beta and gamma activity when the mother is away from the nest for long periods of time (e.g., enhanced beta) compared to when the mother is inside the nest. We also found long-lasting suppression in, beta and gamma activity levels when the infant is nursing or in contact with the nipple, and dramatic surge in power across all frequency bands during milk ejections. Our results demonstrate that during this phase of early development, the maternal caregiver is a primary influence on infant brain activity, with both

transient (seconds) and more long-term (many minutes) effects. Given the important role of neural activity in shaping circuit development and refinement during development, this suggests a powerful direct maternal influence on brain development. Quality of care may be one factor imposing variability of brain circuit development and function.

Disclosures: E.C. Sarro: None. **R.M. Sullivan:** None. **D.A. Wilson:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 221.04/B12

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

Support: Kilian J. and Caroline F. Schmitt Program

Title: Adolescent rat amygdala contains an actively dividing cell population that is positive for doublecortin but not NeuN

Authors: *M. L. SAUL[1], D. L. HELMREICH[2], J. L. FUDGE[1,2];
[1]Neurobio. and Anat., [2]Psychiatry, Univ. of Rochester, Rochester, NY

Abstract: The amygdala is part of the limbic system circuitry that mediates emotional learning and social behavior. These functions undergo tremendous development during adolescence, suggesting changes in amygdala organization. Our previous work showed that dividing cells, marked by bromodeoxyuracil (BrdU), are present in the amygdala of both adolescent (post natal day (PND) 30) and adult (PND 62) male Sprague-Dawley rats. Further characterization of these cells using double-labeling techniques and confocal microscopy showed that BrdU and doublecortin (DCX) double-labeled cells make up approximately 30% of dividing cells in the amygdala in both adolescence and young adulthood. In the present study, we aimed to determine if the BrdU+ cells were migrating into or dividing within the amygdala, and if BrdU/DCX+ cells in the amygdala eventually express the mature neuronal marker, NeuN. Adolescent male rats were given BrdU injections (200 mg/kg x 4) and sacrificed at one of five time points after final injection (24 hours, 5, 10, 28 or 56 days) to determine when BrdU+ cells were present in the amygdala, and if they co-expressed DCX or NeuN. Time points were based on hippocampal studies showing peak BrdU and BrdU/DCX cell numbers at 7-10 days and the emergence of NeuN at 28 days. BrdU+ cells were found in peak numbers in the amygdala at 24 hours and decreased across time points to their lowest level at 56 days ($p < 0.05$). In this population we again found a subset of cells, approximately 30%, that are BrdU/DCX+ double-labeled. These cells were also observed at peak numbers at 24 hours, and decreased to their lowest point at 56

days ($p < 0.05$). Notably, while both cell populations decreased as time progressed, the percentage of total BrdU+ cells that were DCX+ were not significantly different across time. To examine if BrdU cells differentiated into mature neurons, we looked for BrdU/NeuN double-labeled cells. No BrdU/NeuN+ cells were present at either the 24 hour or 28 day time points. These data suggest that cells are actively dividing in the adolescent amygdala. These dividing cells do not increase in number between 24 hours and 5 to 10 days, as in the hippocampus, suggesting a different cell division timeline compared to what is known in the hippocampus. The observed decrease in cells across time is probably due to cell death or dilution of BrdU after repeated cell division. Additionally, the normal decrease in the number of dividing cells across time does not preferentially favor cells that are DCX+. BrdU/NeuN+ cells were not seen at 28 days, as expected based on hippocampal studies, suggesting that at least up until 28 days the cells that divide in the amygdala are not fated to become mature neurons.

Disclosures: M.L. Saul: None. **D.L. Helmreich:** None. **J.L. Fudge:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 221.05/B13

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

Support: Donders institute

Title: Fluoxetine exerts age-dependent effects on myelination

Authors: *Y. KROEZE[1,2], D. G. A. PEETERS[1,2], H. ZHOU[3,4], J. R. HOMBERG[1,2];
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Abstract: The selective serotonin reuptake inhibitor (SSRI) fluoxetine is widely prescribed for treatment of depression and anxiety disorders. The safety of fluoxetine for adults is well established, but adverse outcomes, such as autism- and anxiety-like manifestations, have been reported in prenatally SSRI exposed children and rats. Strikingly, fluoxetine treatment is, amongst others, used to reduce autism- and anxiety-related symptoms during adulthood, suggesting that prenatal versus adult SSRI exposure is associated with opposing outcomes. The mechanisms underlying these age-dependent effects of SSRIs are unknown. Because there is increasing evidence that fluoxetine treatment during adulthood exerts therapeutic effects through neurotrophic mechanisms (For review see Kroeze et al., 2012), we hypothesize that early-life

fluoxetine exposure will target similar pathways but has opposite effects. To test this hypothesis we performed genome-wide gene expression studies on hippocampus tissue of rats treated with fluoxetine at adulthood and rats prenatally exposed to fluoxetine. Gene expression profiling was performed by RNA-seq, and RT-qPCR validations were performed in independent samples. We show that fluoxetine has an effect on genes involved in myelination and that these myelin-linked genes are regulated in opposite direction between early life fluoxetine exposure and fluoxetine treatment during adulthood. Genes involved in myelination are down-regulated after prenatal fluoxetine exposure and up-regulated after adult treatment. One of these genes is ciliary neurtrophic factor (*Cntf*), a survival factor for neurons and oligodendrocytes, which induces myelination of axons (Stankoff et al., 2002; Nash et al., 2011) and mediates neurogenesis (Yang et al., 2008). These results could give us new insides into the mechanisms underlying the opposing effects of early-life and adult fluoxetine exposure on autism and anxiety-like behaviour. As the level of myelination is essential for communication between neurons and therefore brain regions, changes in the expression of genes that regulate myelination could be an important factor by which fluoxetine influences functional brain connectivity, long-term behavior and disease risk.

Disclosures: Y. Kroeze: None. **H. Zhou:** None. **J.R. Homberg:** None. **D.G.A. Peeters:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 221.06/B14

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

Support: NS23805

Title: Fewer accumbens neurons projecting to the ventral tegmental area in adolescent versus adult rats is not a simple reflection of fewer accumbens neurons in adolescents

Authors: *K. P. PARSLEY, R. A. REICHARD, D. S. ZAHM, L. YETNIKOFF;
Pharmacol. and Physiological Sci., St. Louis Univ. Sch. of Med., Saint Louis, MO

Abstract: The mesocorticolimbic dopamine (DA) system has long attracted the interest of researchers concerned with the unique gamut of behavioral and mental health vulnerabilities associated with adolescence. Accordingly, the development of the mesocorticolimbic system has been studied extensively, but almost exclusively with regard to DAergic output. To the contrary, the ontogeny of inputs to the ventral tegmental area (VTA), the source of mesocorticolimbic DA, has been neglected. This is not a trivial oversight, as the activity of VTA neurons, which reflects

their capacity to transmit information about salient events, is sensitively modulated by inputs. To address this issue, we recently compared the organization of VTA afferents in adolescent and adult rats and found fewer inputs to the VTA from an interconnected network of cortical and ventral striatopallidal forebrain structures during adolescence as compared to adulthood (Yetnikoff et al. Soc Neurosci Abst 2012: 790.05). Strikingly fewer VTA-projecting neurons were observed in the adolescent as compared to adult nucleus accumbens (Acb). The present study was undertaken to evaluate the possibility that this observation is a simple reflection of fewer neurons in the adolescent Acb. Groups of four adolescent (postnatal day 39) and four adult (> postnatal day 60) rats were perfused and the brains were sectioned at 50 μm. Three and five adjacent series of sections from the adolescent and adult brains, respectively, were collected. One series of sections from each brain was mounted in rostrocaudal sequence and Nissl stained. The numbers of neurons in the Acb were estimated by the optical fractionator method with the aid of the StereoInvestigator hardware-software platform (MBF Bioscience, Williston, VT). The StereoInvestigator Cavalieri probe was used to assess the volume of the Acb. Differences in numbers or density of neurons in the Acb or Acb volume were not observed. The results indicate that the protracted maturation of afferent connections to the VTA from the Acb is not due to fewer neurons in the Acb during adolescence as compared to adulthood.

Disclosures: K.P. Parsley: None. **R.A. Reichard:** None. **D.S. Zahm:** None. **L. Yetnikoff:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 221.07/B15

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

Support: CNPq

CAPES

FAPERJ

Title: Combined exposure to tobacco smoke and ethanol during adolescence leads to short- and long-term modulation of anxiety-like behavior

Authors: *M. CORREA SANTOS[1], A. MANHAES[2], C. FILGUEIRAS[2], C. CAVINA[3], V. NAIFF[2], A. CARVALHO[2], Y. VILLACA[2];

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Abstract: Tobacco smoking is frequently associated with alcohol drinking and consumption of both drugs typically begins during adolescence. Since anxiety is considered a relevant factor for both smoking and drinking due to its motivating force for a continued consumption, anxiety alterations shared by these two drugs could explain their co-use and co-abuse. Here, we investigated the short- and long-term effects of adolescent tobacco smoke and/or ethanol exposure on anxiety levels. From postnatal day 30 to 45, Swiss mice were exposed to tobacco smoke (SMK - whole body exposure, 8 h/day) and/or ethanol (ETOH - 25% solution, 2 g/kg i.p. injected every other day) as follows: 1) SMK+ETOH exposure; 2) SMK exposure; 3) ETOH exposure; 4) Control. Anxiety levels were assessed with the elevated plus maze (EPM) and open field (OF) tests. By the end of exposure, SMK female mice presented an anxiolytic response in the EPM and this response was intensified by co-exposure to ethanol. A short-term deprivation from SMK elicited an anxiogenic state in females in the EPM. Although neither smoke nor ethanol effects persisted one month post-exposure, SMK+ETOH male and female mice exhibited an anxiogenic response in the OF. Adolescent female mice are more susceptible to the anxiolytic effects of SMK. The stronger effect in SMK+ETOH group suggests that, in females, the combined leads to lower anxiety levels. Anxiety levels do not seem to be relevant during a short-term SMK+ETOH deprivation, however, increased anxiety during long-term smoking and drinking deprivation demonstrate late-emergent effects both in males and females.

Disclosures: M. Correa Santos: None. **A. Manhaes:** None. **C. Filgueiras:** None. **C. Cavina:** None. **V. Naiff:** None. **A. Carvalho:** None. **Y. Villaca:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 221.08/B16

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

Support: NIH Grant R01NS064135

Title: Postsynaptic effects of Cajal-Retzius cells onto interneurons and pyramidal cells of the hippocampal formation

Authors: *G. QUATTROCOLO, G. MACCAFERRI;
Physiol., Northwestern Univ., Chicago, IL

Abstract: The computational functions of hippocampal Cajal-Retzius cells have long remained elusive because of the lack of data on their anatomical and functional connectivity.
We have decided to address this question directly, by genetically driving the expression of a channelrhodopsin variant in hem-derived Cajal-Retzius cells, and by studying the postsynaptic effect of their optogenetic activation on a variety of target neurons.
We initially verified that short pulses (1 ms) of blue light were able to trigger action potentials in Cajal-Retzius cells recorded under either cell-attached (n=9) or whole cell configurations (n=11). The direct measurement of light-induced currents in Cajal-Retzius cells held at -60 mV in voltage-clamp revealed amplitudes of ~50 pA (n=8).
When we examined the effects of their optogenetic activation on potential postsynaptic target cells, we found that both NBQX- and D-AP5-sensitive currents (n=3 and 3, respectively) were routinely detected on stratum lacunosum-moleculare neurogliaform interneurons, with local dendritic and axonal arborizations. The amplitude of the NBQX-sensitive current measured at a holding potential of -60 mV was ~100 pA, similar to the amplitude of the D-AP5 current recorded at +60 mV. Furthermore, preliminary results suggest that optogenetic stimulation of Cajal-Retzius cells can lead to action potential generation in interneurons.
In contrast, when recording from CA1 pyramidal neurons, very rarely did we observe any type of monosynaptic response (only in one case out of ten recording).
In summary, our results suggest the following conclusions: first, that the major neurotransmitter released by Cajal-Retzius cells is glutamate, and, second, the presence of a strong cell type-specificity of their output. Our findings predict that Cajal-Retzius cell activity is likely to be involved in the development of the synaptic dialogue between the entorhinal cortex and the hippocampus.

Disclosures: G. Quattrocolo: None. **G. Maccaferri:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 221.09/B17

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

Support: MH087978 and MH091372

NIH NIAAA IRP

Title: Suboptimal maternal diet alters D1R binding in the offspring brain

Authors: *P. K. THANOS[1], J. ZHUO[2], M. ANANTH[2], R. KIM[2], N. GRISSOM[3], B. GEORGE[4], N. VOLKOW[5], T. M. REYES[4];
[1]Med. Dept, NIAAA / NIH and Brookhaven Natl. Lab., UPTON, NY; [2]Stony Brook Univ., Stony Brook, NY; [3]Univ. of Pennsylvania, Philadelphia, PA; [4]U of Penn, Philadelphia, PA; [5]NIH, Bethesda, MD

Abstract: Suboptimal maternal diet can negatively impact neurodevelopment. Maternal obesity can predispose to both offspring that are large for gestational age (LGA) via overnutrition, and offspring that are small for gestational age (SGA) via placental dysfunction. In humans, both LGA and SGA are biomarkers for an increased risk of multiple mental disorders, particularly Attention Deficit/Hyperactivity Disorder and schizophrenia. Our lab has previously shown that mouse models of LGA (maternal high fat diet) and SGA (maternal low protein diet) produce offspring with profound alterations in mesocorticolimbic gene expression regulating dopamine and opioid function, indicating that the development of these brain regions is particularly vulnerable to gestational insults. Importantly, these two maternal diets affected dopamine and opioid systems in opposing directions (e.g., SGA mice are generally hyperdopaminergic with reduced opioid expression, and the reverse is found for the LGA offspring). These data suggest that specific adverse experiences in gestation can drive dissociable dysfunction in transcription within the same brain regions, but do not establish whether these transcriptional changes are reflected in receptor function.
In this study control, SGA and LGA offspring were generated from dams fed control, low protein or high fat diet, respectively, throughout pregnancy and lactation. At weaning, mice were placed on the control diet and were sacrificed at 12 weeks of age. Brains were flash-frozen, sectioned and D1R autoradiography (Tarazi et al., 1997) was performed using [3H] SCH 23 390. D1R binding was measured in the following brain regions: olfactory tubercle (Tu), nucleus accumbens shell (AcbSh), Acb core, dorsal medial caudate putamen (DM CPu), dorsal lateral CPu (DL CPu), ventral medial CPu (VM CPu), ventral lateral CPu (VL CPu), DL CPu tail, VL CPu tail, and substantia nigra. Additional analyses on mu-opioid receptor and dopamine transporter binding in the same regions are ongoing and will be presented at the meeting. Results showed that the SGA mice (males and females) had significantly higher D1R binding than the standard chow control group in the Tu (20%), Acb (24%), and CPu (23%), while no differences were observed between the LGA and control mice. In conclusion, SGA but not LGA offspring show significantly elevated D1R level in the brain thus affecting DA signaling. These findings advance the current understanding of the how suboptimal gestational diets can adversely impact neurodevelopment and increase the risk for disorders such as ADHD, obesity and addiction.

Disclosures: P.K. Thanos: None. **M. Ananth:** None. **J. Zhuo:** None. **R. Kim:** None. **N. Grissom:** None. **B. George:** None. **N. Volkow:** None. **T.M. Reyes:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 221.10/B18

Topic: A.01. Brain Patterning

Support: NFR Grant

Title: The role of structural parietal characteristics in visuospatial development in pre- and primary-school children: Contributions of retrosplenial cortex

Authors: *S. K. KROGSRUD[1], C. TAMNES[2], H. GRYDELAND[2], J.-L. CHEPKOECH[2], A. FJELL[2], L. MORK[2], K. WALHOVD[2];
[1]Dept. of Psychology, Res. Group For Lifespan Changes In Brain and Cognition, Oslo, Norway; [2]Dept. of Psychology, Res. Group for Lifespan Changes in Brain and Cognition, Oslo, Norway

Abstract: Visuospatial abilities are known to rely heavily on parietal brain structures, and both show early developmental changes. However, the role of structural brain characteristics in development of visuospatial skills is largely unknown. We investigated performance on visuospatial span and visuospatial construction tasks, and cortical thickness in inferior and superior parietal, precuneus, retrosplenial and posterior cingulate regions in a sample of 342 children aged 4-9 years. Measures used were structural magnetic resonance imaging (MRI), Wechsler Memory Scale (WMS) Spatial Span, Wechsler Preschool and Primary Scale of Intelligence (WPPSI) Block Design, and Wechsler Abbreviated Scale of Intelligence (WASI) Block Design scores. For the WPPSI Block Design and WASI Block Design analysis, two different samples were used (<6.5 years of age and ≥6.5 years of age, respectively). Linear regression analysis controlling for sex showed a significant ($p<.001$) effect of age on all three behavioral measures; WMS Spatial Span ($N=346$, $\beta=.68$), WPPSI Block Design ($N=240$, $\beta=.70$), and WASI Block Design ($N=98$, $\beta=.40$). A general linear model analysis with hemisphere, ROIs, age and sex, showed no significant hemisphere main or interaction effects, so mean values of the left and right cortical thickness were used. Regressing out the effect of age and sex, the results showed significant relationships between WMS Spatial Span and retrosplenial thickness ($\beta=-.18$, $p=.018$), significant relationships between WPPSI Block Design and inferior parietal ($\beta=-.14$, $p=.039$), superior parietal ($\beta=-.19$, $p=.006$), and precuneus ($\beta=-.19$, $p=.006$) corties, and a significant relationship between WASI Block Design and retrosplenial cortex ($\beta=-.22$, $p=.045$). Thus, in all instances, better visuospatial task performance was associated with thinner parietal cortices. The results indicate structural maturational effects of medial and lateral parietal cortices on visuospatial abilities among young children.

Disclosures: S.K. Krogsrud: None. **C. Tamnes:** None. **H. Grydeland:** None. **J. Chepkoech:** None. **A. Fjell:** None. **L. Mork:** None. **K. Walhovd:** None.

Poster

221. **Limbic System Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 221.11/B19

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: DOD AR120066 Idea Development Award

Title: Placental tryptophan metabolic dysfunction: a potential pathway for the developmental programming of mental disorders

Authors: *N. GOEDEN[1], A. BONNIN[2];
[2]Cell and Neurobio., [1]USC, Los Angeles, CA

Abstract: Serotonin (5-HT) has been increasingly implicated as a critical modulator of neurodevelopmental processes, from cell proliferation and migration, to later developmental events such as axon guidance and circuitry formation. Depending on the gestational timing, even modest disruptions of 5-HT signaling can have profound effects on the developing fetal brain that persist throughout development and adult life. Recent evidence indicates that the placenta is directly responsible for the synthesis and delivery of 5-HT to the fetus during critical periods of development, leading to the possibility that genetic and environmental perturbations affecting placental tryptophan metabolism may evoke a host of neurodevelopmental abnormalities in the fetal brain, ultimately contributing to the developmental origin of neuropsychiatric disorders. We investigated this issue using a variety of molecular techniques, including a novel ex-vivo placental perfusion system. We demonstrate that maternal exposure to the immunostimulants lipopolysaccharide (LPS), or polyinosinic:polycytidylic acid (poly I:C), induce differential changes in gene expression of enzymes responsible for 5-HT metabolism and synthesis from maternally derived tryptophan in the placenta and fetal brain. Changes in gene expression in the fetus and placenta were observed as quickly as 24 hours after maternal exposure to LPS. Additionally, these changes are spatially quantified both in the placenta and in the fetal brain through the use of a novel in situ hybridization based technology. Furthermore, through HPLC measures of placental and fetal brain tissues, as well as measures of enzymatic activity following immunostimulant exposure, we demonstrate that several key tryptophan metabolites are disrupted by maternal exposure to LPS or poly I:C. Our results indicate that maternal exposure to

infectious agents may lead to disruption of placental 5-HT synthesis, which can adversely impact neurodevelopmental processes in the fetal brain.

Disclosures: N. Goeden: None. **A. Bonnin:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 222.01/B20

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant NS077989

Title: Dynamic regulation of nicotinic synaptic transmission by postsynaptic Group I mGluRs

Authors: *V. RUPPRECHT, Y.-G. SUN, M. BEIERLEIN;
Dept. of Neurobio. and Anat., UTHealth/ Univ. of Texas Med. Sch., Houston, TX

Abstract: Activation of postsynaptic mGluRs in neurons throughout the brain triggers distinct forms of short- and long term plasticity at both glutamatergic and GABAergic synapses. However, a possible mGluR dependent regulation of cholinergic synaptic signaling has never been investigated. We have recently shown that stimulation of cholinergic afferents from the basal forebrain and the brainstem generates biphasic postsynaptic responses in neurons of the thalamic reticular nucleus (TRN), mediated by the activation of both α4β2 nicotinic and M2 muscarinic receptors. To examine a possible role of Group I mGluRs in controlling cholinergic synaptic strength, we isolated cholinergic synaptic inputs to TRN neurons in thalamocortical slices of mice (P13- 16), by pharmacologically blocking fast glutamatergic and GABAergic synaptic transmission. Bath application of the Group I mGluR agonist DHPG led to a long-term potentiation (LTP) of isolated nicotinic EPSCs (nEPSCs) compared to control (174± 9%, n=12), while muscarinic IPSCs remained unchanged (102± 5%, n= 6), indicating a postsynaptic expression. DHPG-induced LTP of nEPSCs required postsynaptic calcium increases and the activation of phospholipase C (PLC). In addition, LTP induction required the synergistic activation of both mGluR1 and mGluR5, as application of either the mGluR1 antagonist CPCCOEt or the mGluR5 antagonist MPEP eliminated LTP. Interestingly, CPCCOEt application led to a reduction of nEPSCs (76 ± 7%, n= 5), while MPEP had no significant effect (96 ± 11%, n= 6), indicating a persistent activation of mGluR1 under baseline conditions. Bath application of the glutamate transporter blocker TBOA led to a transient increase in nEPSC amplitude (175± 22%, n=5), blocked by either CPCCOEt or MPEP, suggesting that mGluRs are sensitive to increases in ambient glutamate levels. Taken together, our data show that the

synergistic activation of mGluR1 and mGluR5 can lead to both short- and long-term increases in nicotinic synaptic transmission.

Disclosures: V. Rupprecht: None. **Y. Sun:** None. **M. Beierlein:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 222.02/B21

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant 5T32MH067564-08

NIH Grant 5R21MH085117-02

BRF Grant SG 2010-13

Title: Cholinergic modulation of neocortical pyramidal neurons

Authors: *T. HEDRICK[1], J. WATERS[2];
[1]Physiol., Northwestern Univ, Fienburg Sch. of Med., CHICAGO, IL; [2]Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL

Abstract: Acetylcholine (ACh) shapes cortical function during sensory perception, motor control, arousal, attention, learning and memory. Cortical cholinergic dysfunction is tied to conditions such as Alzheimer's disease. We investigated postsynaptic responses of neocortical pyramidal neurons to synaptic ACh release.
We expressed channelrhodopsin-2 in cholinergic neurons in nucleus basalis and axons in cortex. To evoke ACh release in acute slices, we stimulated cholinergic axons via widefield illumination with a blue LED. In whole-cell recordings from pyramidal neurons, ACh release evoked muscarinic ACh receptor (mAChR) and nicotinic AChR (nAChR)-mediated potentials. In this study we focused on the nAChR component.
nAChR-mediated responses had an amplitude of 2.6 ± 0.36 mV (60 neurons), rise and decay time-constants of 32 ± 5 ms and 161 ± 14 ms (15 neurons), and a latency of 7.2 ± 0.7 ms (15 neurons). nAChR-PSPs were insensitive to mAChR, glutamate and GABA-receptor antagonists (5 of 5, 5 of 5, 8 of 8 neurons, respectively), but were eliminated by nAChR antagonist mecamylamine (11 of 11 neurons). We conclude that the nAChR-mediated response is a monosynaptic nAChR-mediated PSP.
When the neuron was suprathreshold, nAChR activation increased spike frequency. With the

neuron subthreshold, nAChR activation reduced the rheobase by 11.6 ± 3.7 pA and evoked persistent spiking lasting several seconds. Persistent spiking depended upon nAChR activation and a rise in internal calcium, but was unaffected by mAChR, glutamate and GABA receptor antagonists. We show that nAChR-activation can transiently increase pyramidal neuron spiking, or dramatically increase spiking over several seconds via persistent spiking.
nAChR PSPs were evoked in all layers of primary motor, visual and prefrontal cortices, but there were local variations in nAChR PSP occurrence. In visual cortex, nAChR PSPs were common in pyramidal neurons in all layers (layer 2/3: 83%, layer 5: 82%, layer 6: 89%); in prefrontal cortex, common only in layer 6 (layer 2/3: 30%, layer 5: 15%, layer 6: 63%); and in primary motor cortex, common only in deep layers, particularly layer 5 (layer 2/3: 19%, layer 5: 90%, layer 6: 50%).
Presumably, by enhancing pyramidal neuron spiking in some layers, nAChR activation enhances sub-circuits within each of these cortical regions. It is unclear why nAChR activation stimulates pyramidal neurons in different layers in different cortical regions, but presumably ACh serves different functions in these diverse circuits. Our results indicate that ACh enhances cortical circuits via nAChRs on pyramidal neurons, but that the effects of ACh differ significantly between cortical regions.

Disclosures: T. Hedrick: None. **J. Waters:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 222.03/B22

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Fletcher Jones Fellowship

Whittier College Faculty Research Grant

NSF DBI (1126118)

H. F. Lenfest Endowment for Faculty Support (425380/425382)

Title: Effect of chlorpyrifos exposure on the development of cholinergic neurons

Authors: *E. A. FRADINGER[1], G. X. GARCIA[1], J. M. TILGMAN[2], O. MAC[1], A. JIMENEZ[1], D. BOURGAIZE[1], F. WATSON[2];

[1]Biol. Dept., Whittier Col., WHITTIER, CA; [2]Dept. of Biol., Washington and Lee Univ., Lexington, VA

Abstract: Organophosphate pesticides are known to inhibit acetylcholine esterase, an enzyme that degrades acetylcholine at the cholinergic synapse. In this study PC-12 cells, *Caenorhabditis elegans* and *Xenopus leavis*, were used to understand the impact of the organophosphate pesticide chlorpyrifos on the development of the cholinergic nervous system. Chlorpyrifos at concentrations greater than 100µM was lethal to differentiated PC-12 cells, *C. elegans* and *X. leavis*. It was found that PC-12 cells were most susceptible to chlorpyrifos-induced cell death at later stages of development. To understand the *in vivo* effects of chlorpyrifos on developing cholinergic neurons, we used transgenic *C. elegans* and *X. leavis* expressing GFP-labeled cholinergic neurons. *C. elegans* these cholinergic neurons show that exposure to chlorpyrifos at concentrations above 10 µM results in changes to the DA and DB cholinergic ganglia at the L1 stage. Embryos exposed to concentrations above 10 µM do not survive past the L1 stage. The effect of chlorpyrifos exposure on GFP-expressing neurons in *X. leavis* somites will be examined by confocal microscopy to determine whether the number of cholinergic neurons and their ability to form functional synapses is affected. Since many organophosphate pesticides are USDA approved for use in agriculture, knowing their impact on neuronal development is critical.

Disclosures: E.A. Fradinger: None. **G.X. Garcia:** None. **J.M. Tilgman:** None. **O. Mac:** None. **A. Jimenez:** None. **D. Bourgaize:** None. **F. Watson:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 222.04/B23

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Murphy Fellowship

Whittier College Faculty Research Grant

Title: Reactivation of zebrafish acetylcholinesterase by novel oxime compounds following inhibition by organophosphate pesticides

Authors: *H. R. SCHMIDT[1], Z. RADIć[2], P. TAYLOR[2], E. A. FRADINGER[1];
[1]Biol., Whittier Col., Whittier, CA; [2]Skaggs Sch. of Pharm. and Pharmaceut. Sci., UCSD, La Jolla, CA

Abstract: Organophosphates (OPs) are a class of pesticides and nerve agents whose mechanism of toxicity involves irreversible inhibition of acetylcholinesterase (AChE). This leads to hyperstimulation by cholinergic neurons due to a slower catalytic hydrolysis of acetylcholine released at the synaptic cleft. Current antidotal treatment involves the use of oxime reactivators such as 2-PAM, which have limited efficacy *in vivo* due to their inability to cross the blood-brain barrier. Similarly, passage of 2-PAM as a quaternary antidote into the circulation and CNS of fish may be limiting. Here, we examine the suitability of the zebrafish (*Danio rerio*) model system for *in vitro* and *in vivo* tests of antidotal efficacy by comparing the inhibition and reactivation kinetics of purified human AChE with zebrafish AChE isolated from tissue homogenates. Inhibition kinetics for zebrafish AChE was determined for two OPs, chlorpyrifos oxon and dichlorvos. Chlorpyrifos oxon was found to be a more potent inhibitor of AChE activity than dichlorvos. We then compared the zebrafish AChE reactivation kinetics of two tertiary amine containing oximes, to the quaternary antidote, 2-PAM. All three oximes reactivated zebrafish AChE after OP inhibition, enabling us to begin to compare *in vitro* reactivation kinetics with protection from OP toxicity. Data revealed that zebrafish AChE demonstrated similar inhibition and reactivation kinetics to human AChE. Therefore, the zebrafish emerges as an appropriate and convenient model system for the *in vivo* study of novel oxime AChE reactivators.

Disclosures: H.R. Schmidt: None. **E.A. Fradinger:** None. **Z. Radić:** None. **P. Taylor:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

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Program#/Poster#: 222.05/B24

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: H. F. Lenfest Endowment for Faculty Support (425380/425382)

Whittier College Faculty Research Grant

Title: USDA-approved organophospate pesticides, cause developmental abnormalities in two aquatic vertebrate models

Authors: J. GREGG[1], J. M. TILGHMAN[3], H. SCHMIDT[2], *F. L. WATSON[4], E. A. FRADINGER[2];
[1]Dept. of Biol., [2]Dept. of Biolog, Whittier Col., Whittier, CA; [3]Dept. of Biol., Washington and Lee Univ., Lexington, VA; [4]Biol., Washington & Lee Univ., LEXINGTON, VA

Abstract: Acetylcholine esterase (AChE) degrades acetylcholine at the cholinergic synapse and is necessary for proper nerve function. Organophosphate pesticides, widely used in agriculture, act by inhibiting AChE. To evaluate organophosphate toxicity, Danio rario and Xenopus laevis embryos were exposed to chlorpyrifos, dichlorvos, and diazinon at concentrations ranging from 0.1 µM to 1 mM over a 10 day period. In D. rerio, all three pesticides were found to be toxic to developing embryos at concentrations at or above 100 µM. However, disruption of cholinergic activity, observed as increased spontaneous movements, decreased heart rate, and impaired larval swimming ability, was only seen in embryos exposed to chlorpyrifos and dichlorvos. Similarly, day 10 survival of X. laevis embryos decreased to zero or at concentrations of 100 µM of chlorpyrifos and dichlorvos, and toxicity was observed at concentrations of 1 µM and 0.1 µM for chlorpyrifos and dichlorvos, respectively. Gross morphological abnormalities such as spinal curvature and decreases in overall length, swimming ability, and heart rate were observed at and above these toxicity thresholds. Such abnormalities are indicative of disruption of cholinergic activity. In agreement with results for D. rerio, 100 µM diazinon decreased survival at day 10 to near zero, but it did not cause spinal curvature at lower concentrations in X. laevis. Since many organophosphate pesticides are USDA approved for use in agriculture, knowing their impact on aquatic vertebrate species living in run-off areas is critical.

Disclosures: J. Gregg: None. **J.M. Tilghman:** None. **H. Schmidt:** None. **F.L. Watson:** None. **E.A. Fradinger:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 222.06/B25

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Intramural Research Program of the NIH, National Institute of Environmental Health Sciences

Title: Inducing hippocampal network plasticity by coincident septal inputs

Authors: *Z. GU, J. YAKEL;
Lab. of Neurobio., NIEHS/NIH, RTP, NC

Abstract: It is well established that septal cholinergic inputs to the hippocampus play a central role in modifying hippocampal network activities and higher brain functions. However, much remains to be known about the underlying mechanisms. Here we induced hippocampal theta oscillations by co-activation of septal cholinergic inputs and local hippocampal Shaffer collateral

inputs to the CA1 in a septal-hippocampal co-culture system. Moreover, after 3-5 times of co-activation, hippocampal theta oscillations could then be induced by only a single-pulse Shaffer collateral stimulation. This induction of theta oscillations during the co-activation period was blocked by either muscarinic or α7 nicotinic AChR (acetylcholine receptor) antagonists, or by NMDAR antagonists. However after the co-activation period, the theta oscillations could not be blocked by any of the AChR antagonists; they were still completely blocked by NMDAR antagonists. These results suggest that coincident septal cholinergic inputs, through activation of muscarinic and α7 nicotinic AChRs, can induce lasting modifications of hippocampal network connectivity, providing a mechanism by which stable patterned hippocampal network activity can be achieved by local hippocampal inputs.

Disclosures: Z. Gu: None. **J. Yakel:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 222.07/B26

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NINDS K08 to ABN

NINDS RO1 to ACK

Title: Striatal cholinergic interneurons cause disynaptic axoaxonic inhibition of output neurons via dopamine neuron terminals

Authors: *A. B. NELSON[1], N. HAMMACK[2], R. SEAL[3], A. KREITZER[2];
[1]Neurol., Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]Gladstone Inst., San Francisco, CA; [3]Pittsburgh Univ., Pittsburgh, PA

Abstract: As the input nucleus of the basal ganglia, the striatum integrates sensorimotor information and feedback about the rewarding or aversive outcomes of actions, allowing it to participate in action selection. Striatal cholinergic interneurons are known to have a burst-pause-burst firing pattern in vivo which aligns with salient events such as reward-predictive cues. However, the mechanism by which striatal cholinergic interneurons affect striatal output is not fully understood. Recent experiments have shown that optogenetic activation of cholinergic interneurons in evokes disynaptic inhibitory responses in striatal output neurons. To address the question of how this inhibition is mediated, we have made whole-cell recordings from brain slices containing the striatum, in which we optogenetically stimulate cholinergic interneurons.

Such activation triggers inhibitory responses in striatal output neurons, which is blocked by antagonists of nicotinic receptors, and can be reconstituted by puffing carbachol directly onto striatal output neurons. We also have found evidence that these inhibitory responses are mediated axoaxonic neurotransmission through nigrostriatal dopamine terminals, as it can be blocked by toxin-mediated killing of dopaminergic neurons, or reversible inhibition of synaptic vesicle loading in these neurons. We propose that optogenetic activation of cholinergic interneurons leads to activation of nicotinic receptors on the terminals of dopamine neurons, which in turn release GABA onto striatal output neurons, causing inhibitory synaptic responses. These results suggest an intimate relationship between striatal cholinergic interneurons and nigrostriatal dopamine neurons, both of which signal reward-related cues and can profoundly influence striatal output.

Disclosures: A.B. Nelson: None. **N. Hammack:** None. **R. Seal:** None. **A. Kreitzer:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 222.08/B27

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: OTKA K101326

OTKA K83710

OTKA K100722

FP7/2007-2013 Grant Agreement 245009

NIH/NINDS grant NS023945 (LZ)

Title: Morphological evidence for direct glycinergic input to cholinergic neurons in the mouse basal forebrain

Authors: Z. BARDÓCZI[1], M. WATANABE[2], L. ZÁBORSZKY[3], Z. LIPOSITS[1,4], *I. KALLO[1,4];
[1]Dept. of Endocrine Neurobio., Inst. Exptl. Med. - HAS, Budapest, Hungary; [2]Dept. of Anat., Hokkaido Univ. Sch. of Med., Sapporo, Japan; [3]Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ., Newark, NJ; [4]Dept. of Neuroscience, Fac. of Information Technol., Pázmány Péter Catholic Univ., Budapest, Hungary

Abstract: Glycine is a major inhibitory neurotransmitter in the mammalian central nervous system and an important regulator of glutamate neurotransmission; its extracellular concentration is regulated by glycine transporters (GLYT-1 and GLYT-2), which are present in the cell membrane of a subset of glial cells and neurons. Glycine transporter positive glial cells and neuronal processes have been detected in basal forebrain areas rich in cortically projecting cholinergic neurons. Cholinergic neurons have been implicated in cortical activation, attention, motivation, memory, and show demise in neuropsychiatric disorders, including Alzheimer's disease. The aim of the present study was to reveal whether or not cholinergic neurons in the basal forebrain are in morphological relationship with GLYT1- and/or GLYT2-immunoreactive (IR) cells. Double-label immunohistochemical methods were used to detect either of the transporters and the cholinergic neuronal marker choline acetyltransferase (ChAT) in mouse brain sections. Distinct fluorescent or electron dense markers were employed, respectively, for subsequent confocal or electron microscopic analyses. Under fluorescence microscope, strong GLYT1-immunoreactivity was identified in the medial septum (MS), vertical and horizontal diagonal bands of Broca (VDB and HDB), ventral pallidum (VP) and substantia innominate (SI). A striking overlap was observed with ChAT-IR neurons in most major areas where cholinergic neurons occur. At the ultrastructural level, GLYT-1-immunoreactivity was detected in glial elements; in some cases these GLYT-1-IR structures were found in close proximity to cholinergic dendrites and cell bodies receiving unlabeled synaptic inputs. Immunoreactivity for GLYT-2 (the marker protein of glycinergic neurons) was detected in varicose axons. Confocal microscopic analysis of the different subpopulations of ChAT-IR neurons demonstrated appositions among GLYT-2-IR axon varicosities and cholinergic cell bodies and dendrites. Appositions in the HDB were further studied at the electron microscopic level and symmetric and asymmetric synapses were identified between glycinergic axons and ChAT-IR neurons. Our results suggest that glycine have a direct influence on basal forebrain cholinergic neurons by demonstrating (1) synaptic connections between glycinergic axons and cholinergic neurons and (2) strong presence of glial and neuronal transporters in the vicinity of cholinergic neurons capable to tightly regulate extracellular levels of glycine. Identification of the participating receptor mechanisms, however, needs further studies.

Disclosures: Z. Bardóczi: None. **M. Watanabe:** None. **L. Záborszky:** None. **Z. Liposits:** None. **I. Kallo:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 222.09/B28

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIGMS Grant 1K12GM102778

NIH 1 DP1 OD007014

NIH R01 NS 22061

Title: Optogenetic analysis of basal forebrain cholinergic neurons: Comparison of wild-type and type III neuregulin 1 mutant mice

Authors: *G. Y. LOPEZ, D. A. TALMAGE, L. W. ROLE;
Stony Brook Univ., Stony Brook, NY

Abstract: Deficits in acetylcholine (ACh) transmission have been related to some of the cognitive and behavioral impairments associated with neurodegenerative and neuropsychiatric diseases. It has been difficult to dissect the physiological functions of ACh, mainly due to the sparse nature of cholinergic neurons and the broad and diffuse distribution of their projections. A principal source of cholinergic neurons projecting to the cortex is the nucleus basalis of Meynert (NBM). We use gene delivery of a viral vector (AAV1-DIO-oChIEF-dsTomato) and the Cre-lox system to express the light-activated protein ChIEF in NBM cholinergic neurons of a ChAT-Cre/eGFP-Tau mouse line. Whole-cell patch clamp recording in acute brain slices and optogenetic stimulation were combined to examine the effects of selective activation of NBM cholinergic neurons. We first obtained an electrophysiological profile of cholinergic neurons in the NBM and compared their electrical properties to non-cholinergic neurons. A prominent difference between cholinergic and non-cholinergic neurons was the lower firing frequency of cholinergic neurons during depolarizing current injection (13.5 ± 2.6 Hz vs. 53.2 ± 9 Hz). The electrical properties of cholinergic neurons expressing ChIEF channels were not significantly different from control cholinergic neurons. Light-evoked action potentials and inwards currents were evident following train stimulation in cholinergic neurons. ChIEF+ cholinergic neurons were able to follow trains of opto-stimulation with increasing frequencies, 1 - 20 Hz. However, the percent efficiency in following opto-stimulation decreases with increased frequency of stimulation (< than 20 % at 20 Hz). To examine possible alterations in NBM neurons and cholinergic circuits in a genetic mouse model of schizophrenia we are now extending our work to type III neuregulin 1 mutant mice. The knowledge gained from my studies will advance our understanding of ACh actions in health and disease.

Disclosures: G.Y. Lopez: None. **D.A. Talmage:** None. **L.W. Role:** None.

Poster

222. **Acetylcholine**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 222.10/B29

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: VEGA 1/1139/12

SK-FR-0048-11

Université Paris Descartes

AFM

ANR maladies rares

Stefanik 26367RK

Title: Cholinergic system in brain is extremely adaptable

Authors: *A. HRABOVSKA[1], E. KREJCI[2];
[1]Fac. of Pharmacy, Comenius Univ., Bratislava, Slovakia; [2]CESeM, CNRS UMR 8194, Univ. Paris Descartes, Paris, France

Abstract: Cholinergic system in brain is believed to be essential for many physiological functions, especially cognitive and motor functions. Its impairment has been described in some severe neurological pathologies, e.g., Alzheimer's disease, Parkinson's disease and Huntington's disease. We generated a mouse model in which cholinergic system in brain is disturbed. By deletion of gene for proline-rich membrane anchor (PRiMA) which organizes and attaches tetramers of acetylcholinesterase (AChE) to the outer neuronal membrane, 95% of AChE activity is removed from brain. This results in 300-fold increase in the level of acetylcholine. Nevertheless, mutant mice do not develop any obvious change in the phenotype. They are undistinguishable from wild-type littermates. Detailed comparative behavioral studies do not show any significant change in learning or memory capabilities. Only subtle motor deficits are observed in mutant mice2. The phenotype seems to be preserved due to sever downregulation of muscarinic and mild downregulation of nicotinic receptors. In contrast, genetic manipulations resulting in removal of AChE activity at the periphery (with or without changes of AChE activity in CNS) severely change the phenotype. Our results suggest extreme plasticity of cholinergic system in CNS.
1 Dobbertin et al. 2009; 2 Farar et al. 2012.

Disclosures: A. Hrabovska: None. **E. Krejci:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.01/B30

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant 067310

Title: Molecular physiology of the behavioral switch to ecdysis in *Drosophila* melanogaster

Authors: D.-H. KIM[1], M. HAN[2], Y.-J. KIM[2], *M. E. ADAMS[1];
[1]Univ. of California, Riverside, CA; [2]Life Sci., Gwangju Inst. of Sci. and Technol., Gwangju, Korea, Republic of

Abstract: Ecdysis, the shedding of old cuticle at the end of each molt in insects, occurs through performance of an innate, chemically-coded behavioral sequence. In Drosophila, ecdysis triggering hormones (ETH) initiate and schedule three distinctive behavioral subunits at pupation - pre-ecdysis, ecdysis, and post-ecdysis. ETH initiates and schedules the stereotypic sequence through direct and sequential activation of peptidergic ETH receptor (ETHR) ensembles in the central nervous system (CNS). We examined functional roles of individual ETHR ensembles in the switch from pre-ecdysis to ecdysis behavior by systematic ablation and electrical inactivation. The minimal peptidergic circuit necessary for the behavioral switch from pre-ecdysis to ecdysis consists of central neurons that co-release the peptides CCAP, Ast-CC, MIP, and bursicon. Targeted activation of this minimal circuit via temperature-sensitive TRPM8 channels initiates ecdysis behavior, but recruitment of additional CCAP neurons is necessary for its full expression. Timing of the switch to ecdysis can be altered in predictable ways by changing ETHR density in bursicon neurons: reduction in ETHR expression delays the switch to ecdysis, while increased ETHR expression accelerates it. Similarly, the level of ETHR expression alters the timing of ETH-induced calcium dynamics in bursicon neurons. Manipulation of G protein function in bursicon neurons suggests they receive both excitatory and inhibitory inputs, and that disinhibition determines precise timing of the switch to ecdysis.

Disclosures: D. Kim: None. **M. Han:** None. **Y. Kim:** None. **M.E. Adams:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.02/B31

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSERC Grant 46292

NSERC PGS 426919

Title: Cell-specific modulation by octopamine and proctolin in *Drosophila* melanogaster

Authors: *K. G. ORMEROD[1], L. DEADY[2], A. J. MERCIER[1];
[1]Biol. Sci., Brock Univ., St. Catharines, ON, Canada; [2]Neurosci., Western New England Univ., Springfield, MA

Abstract: The nervous system utilizes a diverse array of signalling molecules to convey information. Drosophila melanogaster contains a large number of substances such as peptides and biogenic amines that act either as transmitters or modulators of synaptic efficacy. Since many of these substances elicit virtually identical effects, they are often thought to act in a functionally redundant manner. We examine an alternative view, that signalling molecules can elicit similar effects but act on specific cells or subsets of cells that may be mutually exclusive. We previously reported that a peptide (DPKQDFMRFamide), which is thought to function as a neurohormone, elicits cell-specific effects on muscle fibers, decreasing input resistance and nerve-evoked contractions to a greater extent in some fibers than others. We now expand our investigations of muscle-cell specific effects of modulatory substances that are thought to be released as co-transmitters onto muscle cells of 3rd instar Drosophila larvae. Octopamine increases EJP amplitude and nerve-evoked contractions to a greater extent in some fibers (12, 13) than others (6, 7) [EJP 10-5 M: 41 + 2.0 and 28 + 3.6% respectively; Muscle Force: 10-5 M: 34.64 + 0.9 and 24.09 + 0.6% respectively]. Proctolin (RYLPT), on the other hand, alters EJP amplitudes in muscle fibers 12 and 13 but has no effect on EJP amplitude in fibers 6 and 7. These observations indicate that co-transmitters can act on specific sub-sets of muscle fibers. We are currently examining the mechanisms underlying such cell-specificity, and future work will examine the physiological implications of cell-specific actions of co-transmitters at neuromuscular junctions.

Disclosures: K.G. Ormerod: None. **L. Deady:** None. **A.J. Mercier:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.03/B32

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: The influence of GABA on the feeding behavior of the cockroach Rhyparobia maderae

Authors: *E. M. DUBON, R. W. COHEN;
Biol., California State University, Northridge, Northridge, CA

Abstract: The central nervous system in vertebrates and invertebrates displays inhibitory neurotransmission that is primarily achieved by activity of the neurotransmitter GABA and its various receptors. GABA has been shown to be a ubiquitous neurotransmitter in vertebrates and, important to this study, in insects. It has been observed that GABA is primarily found in the insect central and peripheral nervous systems, including the mushroom bodies, a prominent region for behavioral regulation. Previous research has indicated that GABA inhibits insect muscle contraction when GABA receptor-linked chlorine channels hyperpolarize the resting membrane potential. GABA has also been shown as having a prominent role in controlling learning and memory in the cockroach olfactory system. Our lab focuses on the neurotransmitter regulation of feeding behavior in insects; specifically how certain neurotransmitters (serotonin, octopamine and dopamine) regulate nutrient self-selection in cockroach nymphs. In this poster, experimentation was begun to examine whether GABA has a direct effect on food consumption and choice in the cockroach *Rhyparobia maderae*. Initially, large cockroach nymphs were selected and placed in a warm incubator with a constant temperature of 28° C and had free access to water but no food. After seven days of starvation, the nymphs were divided equally into experimental and control groups. The experiment nymphs were injected with 1 [[Unsupported Character - Symbol Font ]]l of the effective GABA agonist muscimol (concentration= 1mg/kg). The control group nymphs were injected with an identical volume of insect saline (0.7% NaCl). Immediately after injection, the nymphs were placed in feeding arenas containing water and a choice between pure sucrose and casein cubes. After 24 hours of feeding, cubes were removed, dried in an oven, and weighed to determine the amount eaten. Results indicated that the experimental muscimol group consumed the same amount of casein, but significantly less sucrose compared to the control saline group. In light of these self-selection results, we continue to discover the role of GABA in cockroach food selection, including the use of selected antagonists (picrotoxinin) and the immunostaining of the cockroach brain to detect the exact location of GABA. With these strategies we should establish how GABA affects food choice in *Rhyparobia maderae* nymphs.

Disclosures: E.M. Dubon: None. **R.W. Cohen:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.04/B33

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR Grant MOP111211

Title: Protein kinase C enhances voltage-gated Ca2+ influx and secretory efficacy to facilitate peptide release in neuroendocrine cells

Authors: *C. J. GROTEN[1], N. S. MAGOSKI[2];
[1]Physiol., [2]Queen's Univ., Kingston, ON, Canada

Abstract: Neuroendocrine cells present periodic enhancement of excitability and hormone secretion to initiate fundamental behaviors such as reproduction. Such plasticity is conferred by protein kinase-dependent phosphorylation. A tractable system for studying this process is provided by the bag cell neurons of the marine mollusc, Aplysia californica. Following stimulation, these neuroendocrine cells undergo an afterdischarge, during which egg-laying hormone (ELH) is secreted to trigger reproduction. During the afterdischarge, several kinases are upregulated, including protein kinase C (PKC). An established role for PKC is to initiate the membrane insertion of a separate class of voltage-gated Ca2+ channels; however, the contribution of these channels to peptide secretion remains unknown. In addition to altering Ca2+ influx, PKC has the potential to facilitate hormone release through regulation of the secretory process. To examine the function of PKC in controlling peptide secretion, whole-cell recordings of fura-loaded cultured bag cell neurons were performed, allowing for measurement of secretion, with capacitance tracking under voltage-clamp, and intracellular Ca2+, with fluorescence microscopy. To quantify secretion, membrane capacitance was measured before and after a 1-min, 5-Hz train of depolarizing steps. We initially established that stimulus-induced capacitance changes were related to hormone secretion by ELH knockdown. Treatment of cultured bag cell neurons with ELH dsRNA caused a significant reduction in the magnitude of anti-ELH staining and train-induced capacitance responses. The effects of PKC activation were tested with the diacylglycerol analogue PMA. Treatment with 100 nM of PMA caused a two-fold increase in the train-evoked membrane capacitance response. Preventing PKC-dependent channel insertion by disrupting the actin cytoskeleton, with 10 µM latrunculin, attenuated the PMA effect. To test if PKC influences secretion independently of Ca2+ channel regulation, PMA was applied subsequent to establishing whole-cell recording, which prevents Ca2+ channel insertion. This online PMA treatment significantly increased the stimulus-evoked capacitance

change and the magnitude of secretion to repeated train stimuli. Thus, PKC prepares the bag cell neurons for ELH secretion by enhancing Ca2+ influx and directly regulating the secretory process. Consequently, PKC appears to act as a gate for the transition in neuronal output required for reproduction and species propagation.

Disclosures: C.J. Groten: None. **N.S. Magoski:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.05/B34

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSF- IOS-112095

IOS-0814411

GSU Brains and Behavior Seed Grant

Title: Comparison of serotonin 1a and 2 receptor subtype mRNA expression across tissue, ganglia, and single neurons in the opisthobranch mollusc Tritonia diomedea

Authors: *A. TAMVACAKIS, P. KATZ;
Georgia State Univ., Atlanta, GA

Abstract: Serotonin (5HT) is a neuromodulator that affects several behaviors in the sea slug Tritonia diomedea. Identifying the 5HT receptor (5HTR) subtypes that are involved in these behaviors would aid in the study of their neural mechanisms. Opisthobranch molluscs have five known 5HTR subtypes (Nagakura et al., 2010, J. Neurochem). Serotonergic neurons innervate peripheral tissues to affect sensory and motor behaviors (Moroz et al., 1997, J. Comp. Neurol). Each ganglion of the CNS plays a different role in 5HT-mediated behaviors as do individual identified neurons. We therefore investigated whether these differences were reflected in the expression patterns of two 5HTR subtypes.
Quantitative real-time PCR (qPCR) was used to compare mRNA expression of 5HTR subtypes 1a and 2 in: 1) peripheral tissue [rhinophores, gills, oral veil, and oral groove], 2) whole ganglia [cerebro-pleural, pedal, and buccal ganglia], and 3) single cell-types [the giant serotonergic cerebral cell, pedal neuron 5, and pedal neuron 6].
Preliminary results showed differences in subtype expression across tissue types. In peripheral

tissues, 5HTR1a mRNA expression was higher in the oral groove and rhinophores compared with the oral veil and gills, which showed low 1a expression. 5HTR2 mRNA was relatively highly expression in rhinophore and oral veil, but showed no measurable expression in gill or oral groove tissue. In comparing the CNS ganglia, 5HTR1a mRNA expression was significantly higher in the cerebro-pleural and buccal compared to the pedal ganglia, but all three ganglia exhibited lower 5HTR1a expression compared with oral groove and rhinophore tissue. The different ganglia did not show a significant difference in expression of 5HTR2 mRNA, and were similar to expression levels observed in rhinophore and oral veil tissue. Of the three cell types compared, none showed expression of either 1a or 2 subtypes, although pre-amplification of single-cell mRNA may be necessary for accurate detection of low-copy number transcripts such as 5HTRs.
Identifying 5HTR subtypes expressed in various tissues is important for understanding how neuromodulatory signaling facilitates different behaviors. Differences in 5HTR expression in peripheral tissues may mean that distinct 5HTR subtypes play different roles in sensory and effector systems. Lower expression of 5HTR1a in the pedal compared with other ganglia may indicate that 5HTR1a is not involved in activation of many serotonin-responsive pedal neuron functions. Further study of the other 5-HTR subtypes will provide additional information about the roles of 5-HT in Tritonia.

Disclosures: A. Tamvacakis: None. **P. Katz:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.06/C1

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Electrophysiological properties of ASE neurons of *C. elegans*

Authors: *T. SHINDOU[1], M. OCHI-SHINDOU[1], T. MURAYAMA[2], T. SASSA[2], J. R. WICKENS[1], I. MARUYAMA[2];
[1]Neurobio. Res. Unit, [2]Information Processing Biol. Unit, Okinawa Inst. Sci. Technol., Okinawa, Japan

Abstract: The ASE chemosensory neurons, which are composed of a pair of ASEL and ASER neuron, are the main taste receptors of C. elegans and play a critical role in chemotaxis to salts. The recent studies including optical recordings of calcium imaging have revealed that ASEL

preferentially senses Na+ and serves as ON cell to NaCl, whereas ASER preferentially detects Cl- and acts as OFF cell to NaCl. However, the physiology of ASE neurons to chemotaxis remains unknown and the electrophysiological characterisation of ASE neurons is needed to determine what mechanisms involve responses to chemotaxis. We tried in vivo patch-clamp recording in C. elegans and investigated electrical properties of identified ASE neurons. ASE neurons had a membrane potential of around -60 mV and an input resistance of more than 1 GΩ. Spontaneous small depolarizing events (<5mV amplitude) were observed at a resting membrane potential. Voltage response to depolarising current injections was linearly graded depending on the current intensity, but after reaching around -40 mV, broad and large depolarisations were induced, indicating the nonlinear voltage response to current injections. For comparison, we also recorded AVA neurons (one of command interneurons). AVA neurons had a more silent spontaneous activity than ASE neurons and showed a linear increase of voltage responses by current injections, indicating the graded potentials. The active membrane properties of ASE neurons may contribute to effective signalling as a sensor to NaCl.

Disclosures: T. Shindou: None. **M. Ochi-Shindou:** None. **T. Murayama:** None. **T. Sassa:** None. **J.R. Wickens:** None. **I. Maruyama:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.07/C2

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Molecular mechanisms of peptide modulation of C.elegans motor circuit

Authors: *D. TOUROUTINE[1], R. BHATTACHARYA[2], M. FRANCIS[2];
[1]Univ. of Massachusetts Med. Sch., Worcester, MA; [2]Neurobio., Univeresity of Massachusetts Med. Sch., Worcester, MA

Abstract: Functional connections between neurons of the brain are not static, but instead show remarkable functional plasticity that can be altered through environmental cues, experience, or in the context of specific behaviors. This functional plasticity is often mediated by neuromodulators which activate G-protein coupled receptors to modify neuronal dynamics, excitability, and synaptic efficiency. Neuromodulators include diverse families of neuropeptides. To investigate roles for neuropeptide signaling in the regulation of neural circuit activity, we have been studying neuropeptide actions in motor circuit of the nematode C. elegans. We developed a

transgenic strain harboring a gain-of-function (gf) mutation in a ionotropic acetylcholine receptor (L-AChR) localized to the neuromuscular junction. Expression of L-AChR(gf) in muscle caused prolonged postsynaptic current responses and produced easily identifiable locomotory phenotypes: decreased speed and exaggerated muscle flexures during movement. Using a candidate gene approach, we identified the pro-neuropeptide family member nlp-12 as a strong modifier of the behavioral changes caused by L-AChR(gf) expression. NLP-12 shares greatest similarity to vertebrate cholecystokinin, and is expressed in a single mechanosensory interneuron, DVA. To identify components of the NLP-12 signaling pathway, we overexpressed NLP-12 in DVA neurons. Interestingly, overexpression of NLP-12 (OexNLP-12) caused dramatic changes in movement, suggesting direct actions of this neuropeptide on motor circuit activity. The C. elegans genome encodes two genes that are similar to mammalian cholecystokinin receptors, ckr-1 and ckr-2. Combined mutation of ckr-1 and ckr-2 completely suppressed the effects of NLP-12 overexpression, suggesting the receptors act redundantly. Expression studies indicate that CKR-1 and CKR-2 are expressed throughout the nervous system. We are currently identifying subsets of neurons expressing both receptors and conducting rescue experiments using neuron-type specific promoters to identify the relevant sites of NLP-12 action. Additionally, we are testing how NLP-12 affects signaling in the motor circuit using electrophysiology. The results of these experiments will be presented

Disclosures: D. Touroutine: None. **R. Bhattacharya:** None. **M. Francis:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.08/C3

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Colgate University Research Council

Colgate University Biology Department

Colgate university Neuroscience Program

Title: Gene expression of a serotonin receptor following aggressive behavior in the crayfish, Procambarus clarkii

Authors: *A. J. TIERNEY[1], K. K. INGRAM[2], E. SHOLL[2], M. RICHARDS[1], K. ANDREWS[1], M. WHITE[1];
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Abstract: Serotonin (5-HT) plays a role in aggressive behavior in many taxa, and the receptors that mediate this behavior have been extensively investigated in vertebrates. However, relatively little is known about how 5-HT receptors function to mediate aggression and social status in invertebrate species. We used a well-known model organism, the crayfish *Procambarus clarkii*, to investigate the effects of fighting and changes in social status on the expression of the 5-HT2β receptor. Crayfish were arranged in same-sex size-matched pairs, isolated for seven days, and then allowed to fight for 30 minutes. A clear dominance hierarchy, in which one crayfish (designated the subordinate) persistently retreated from its opponent (designated the dominant), was established in all pairs. Control animals did not interact with a conspecific, but were otherwise treated identically. Fights were video taped and the level of aggression displayed by each pair was quantified. One h (n = 10 pairs) or 48 h (n = 10 pairs) after fighting, the brain and abdominal nerve cords were removed from each animal and separately snap-frozen in liquid nitrogen. We designed intron-crossing primers and probes for 5-HT2β and 18S (control gene). Relative mRNA expression was measured using quantitative rt-PCR and quantified using the ΔΔCt method. Preliminary analysis found no significant differences in mRNA expression in dominant and subordinate animals sacrificed one hour after fighting. However, in brains but not abdominal nerve cords, we found a positive correlation between expression of 5-HT2β mRNA and level of aggression. Also, both dominant and subordinate animals displayed higher levels of 5-HT2β mRNA than did control animals. These findings suggest that aggressive interactions can induce rapid changes in the expression of 5-HT receptors in *P. clarkii*, a change that might have lasting affects on behavior. Ongoing work will determine levels of mRNA expression in tissue taken 48 h after fighting and will quantify mRNA expression of a second 5-HT receptor cloned from *P. clarkii* (5-HT1α).

Disclosures: A.J. Tierney: None. **K.K. Ingram:** None. **E. Sholl:** None. **M. Richards:** None. **K. Andrews:** None. **M. White:** None.

Poster

223. "Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.09/C4

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant GMO63787

Title: The effects of mushroom body lesions on the feeding behavior of the cockroach Rhyparobia maderae

Authors: *F. R. LIBBY, R. W. COHEN;
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Abstract: The effects of neurological cell death or injury may have many downstream effects on the resulting behavior it regulates. Physical disruption of these cellular pathways may have significant behavioral consequences depending on the area of the brain affected by injury. In insects, past studies have shown that the paired mushroom bodies in the protocerebrum are associated with learning and memory, and other higher-level behaviors. Our lab has shown that insects self-select an optimal diet when faced with nutrient choices, and that this self-selection is regulated by specific neurotransmitters (serotonin, octopamine, dopamine) in the brain. But the actual brain location for this nutrient regulation has not yet been discovered in insects. In this study, we wanted to determine if the mushroom bodies were directly involved in food choice by lesioning specific regions of the cockroach brain then examining subsequent feeding behaviors. Past studies utilizing insect brain lesioning have done so blindly using non-surgical, invasive techniques with electrolytic probes. Here, we developed a protocol that permits the animal to survive after physically opening up the head cavity to visually access the brain and lesion the mushroom body. After exposing the cockroach brain, a small, heated acupuncture needle was used to ablate bilaterally the mushroom bodies of the cockroach brain. A control group had their head cavity opened gently touched their mushroom bodies with an unheated needle. To study the effects of mushroom body lesioning on food choice, we lesioned large *Rhyparobia maderae* cockroach nymphs and allowed them to recover for 24 hours with a pellet of rat chow and water provided *ad libitum*. After the recovery period, we removed the food and allowed the cockroaches to fast for three days. At the end of this fasting period, we provided the nymphs with a small cube of casein (protein) and one of sucrose (carbohydrate) and allowed them to feed *ad libitum* for 24 hours. In the lesioned group, the nymphs ate an average of 2.93 mg (casein) and 34.3 mg (sucrose), while the control nymphs ate an average of 5.1 mg (casein) and 86.6 mg (sucrose). The lesioned group also ate at a reduced rate compared to the control group. The lesioned group also showed a distinct difference in nutrient selection: 8% casein versus 92% sucrose for the lesioned nymphs compared to the control group who ate 5.6% casein and 94.4% sucrose. These results indicate that the mushroom body ablations may have an influence in the total amount of food that was eaten as well as alter the nymph's choice of available nutrients.

Disclosures: F.R. Libby: None. **R.W. Cohen:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.10/C5

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: HHMI

Point Loma Nazarene University

Title: Post-embryonic development of serotonergic neurons in a Tenebrionid beetle: Time-course and hormonal dependence

Authors: *R. C. ELSON, J. FREGOSO, R. SMITH, J. SOCH;
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Abstract: During post-embryonic, metamorphic development of the mealworm, *Zophobas morio* (Coleoptera: Tenebrioninae; members of a basal order of holometabolous insects), the pattern of motor and interneurons expressing serotonin-like immunoreactivity (SLI) changes within the terminal abdominal ganglion, a neural center involved in the control of digestive and reproductive organs. In this accessible model system, we tracked the normal time-course of changes in SLI and its sensitivity to exogenous doses of the pesticide pyriproxyfen, a juvenile hormone analog (JHA).

Developmental changes in the number and type of neurons expressing SLI begin in the prepupal phase of the last larval instar and are largely complete by the middle of the pupal stage (i.e., during the formation of the adult). Of two pairs of serotonergic motor neurons that innervate the hindgut in the larva, one pair stops expressing the transmitter in the prepupal phase and regains little SLI thereafter. Of three pairs of interneurons present in the larva, one pair stops showing SLI when the larva becomes a prepupa; at the same time, a new, different pair of interneurons first appears. Up to four more "new" pairs of serotonin-containing interneurons appear progressively during the first half of the pupal stage, completing a segmental pattern. Little change occurs in this established pattern after the adult has emerged.

These developmental changes can be inhibited by applying JHA during the prepupal period, at the time when a large surge (the “prepupal peak”) of ecdysteroid is known to occur. The added JHA must supplement the actions of native juvenile hormone, which is normally present at moderate levels during the ecdysteroid surge. This potent agonist likely inhibits ecdysteroid release, suppressing the surge; at the same time it may antagonize genetic programs of development normally initiated by ecdysteroid at this time. Our findings indicate that balanced, timed hormonal actions are crucial for up- and down-regulating transmitter expression during the development of a set of segmental serotonergic neurons.

Disclosures: R.C. Elson: None. **J. Fregoso:** None. **R. Smith:** None. **J. Soch:** None.

Poster

223. "Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.11/C6

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSF CHE-0957784

NIH R01DK071801

Title: Mass spectral characterization of circadian rhythm-related neuropeptide secretion during light-dark cycle in crustacean via *In vivo* microdialysis

Authors: *Z. LIANG, C. SCHMERBERG, L. LI;
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Abstract: Circadian rhythm, driven by circadian clock, allows biological events to anticipate and adapt to changing environmental conditions. Neuropeptide (NP), as an important class of neuromodulators regulating a wide variety of physiological processes, has an important role in this process. For example, in crustaceans, pigment-dispersing hormone(PDH), which are involved in adaptation of the retina to ambient light changes, have been proven to have altered expression levels at different points of the light-dark cycle. NPs are known to act upon the decapod crustacean stomatogastric nervous system (STNS), which has long been used as a model for the study of neuromodulation and generation of rhythmic firing patterns in the coordinated movements of the muscles of the stomach. Here, we employed a multi-faceted mass spectral (MS) platform to identify and quantify NPs secreted from the pericardial organ (PO) into hemolymph in the light-dark cycle of Jonah crab, Cancer borealis.
In this study, in vivo microdialysis sampling of hemolymph from crabs was conducted to collect samples continuously in12 hr: 12 hr light/dark cycle, and the resulting dialysate was analyzed by MS. Preliminary results revealed the secretion of numerous NPs in the hemolymph, including orcokinin, A-, B- and C-types allatostatin (AST), RFamide (RFa), crustacean hyperglycemic hormone precursor-related peptide (CPRP), crustacean cardioactive peptide (CCAP), and PDH families. Concentration changes for several NPs in the microdialysate were observed. A sharp decrease was observed for PDH soon after the light period was initiated; suggesting the involvement of PDHs in the light to dark transitions. A decrease in CCAP was also detected. Elevation of members of AST-A and -B families were observed toward the end of light period and continued to build within 2 hr after the light turned off, which may indicate an opposite role.

Interestingly, similar changing pattern was also observed for orcokinin. These results support previous studies which indicated that under external stimuli, AST and CCAPs may play important roles in regulating and coordinating the activities of the heart and other organs. No clear changing patterns were observed for NPs from the RFa, AST-C and CRRP families. This comparative peptidomic data aids in the identification of NPs that may be important signaling molecules in coordinating crustacean's circadian clocks, and this platform demonstrate the capability for studying highly dynamic NPs changes under environmental alterations.

Disclosures: Z. Liang: None. **C. Schmerberg:** None. **L. Li:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.12/C7

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Overexpression of TDP-43 inhibits NF-κB activity by blocking p65 nuclear translocation

Authors: *J. ZHU, W. JIA;
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Abstract: TDP-43 (TAR DNA binding protein 43) is a heterogeneous nuclear ribonucleoprotein (hnRNP), its aberrant cleavage and aggregation have been found to be mainly responsible for neurodegenerative diseases recently. Its involvement in nuclear factor-kappaB (NF-κB) pathways has been reported in neuron and microglial cells that are linked to amyotrophic lateral sclerosis (ALS). NF-κB pathway targets more than hundreds of genes that are involved in inflammation, immunity and cancer. In nervous system, it plays an important role in neuroinflammation. However, NF-κB only has functions in nucleus that after transporting from cytoplasm by importinα3 (KPNA4) which could also take TDP-43. In our study, we report that TDP-43 overexpression could block TNF-α induced p65 nuclear translocation dose-dependently that further inhibits p65 transactivation activity. Furthermore, the inhibition by TDP-43 is not through preventing IκB degradation but probably by competing the nuclear transporter-importinα3 (KPNA4) and this competition is dependent on the presence of NLS in TDP-43. Silencing TDP-43 by a specific siRNA also increased p65 nuclear localization upon TNF-α stimulation, suggesting that endogenous TDP-43 may be a default suppressor of NF-κB pathway. The above results indicate that TDP-43 may play an important role in regulating the levels of NF-κB activity by control the nuclear translocation of p65. Our finding that TDP-43

constitutively inhibits NF-κB pathway by blocking nuclear transportation of p65 suggests a novel and important role of TDP-43 in inflammatory response in neurodegenerative diseases.

Disclosures: J. Zhu: None. **W. Jia:** None.

Poster

223. "Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"

Location: Halls B-H

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Program#/Poster#: 223.13/C8

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: ARC Grant DP110100297

Title: Toll-like receptor 4 contributes to opioid ligand binding in brain homogenates

Authors: *J. THOMAS[1], A. A. SOMOGYI[1], K. C. RICE[2], M. R. HUTCHINSON[1];
[1]Univ. of Adelaide, Adelaide, Australia; [2]Natl. Inst. on Drug Abuse, Bethesda, MD

Abstract: In the infancy of opioid binding research, attention was focused directly toward the stereoselective receptors that were discovered to be critical for opioid analgesic responses. However, opioids can paradoxically increase pain sensitivity in humans and rodents leading to hyperalgesia and tolerance. Work by the Hutchinson and Watkins groups has demonstrated that genetic removal of immune receptors such as toll-like receptor 4 (TLR4) significantly reduces the development of hyperalgesia whilst simultaneously increasing anti-nociception. It is thought that opioids bind non-stereoselectively to TLR4 leading to a neuroinflammatory response within the CNS, compromising opioid-induced analgesia and contributing to various unwanted actions. The existing data on the non-stereoselective receptor binding kinetics of opioids is limited and requires further investigation. This study set out to examine the receptor binding kinetics of (-)naloxone in both WT and $TLR4^{KO}$ animal brain homogenates and to determine whether further cell base assays are warranted. Wildtype (WT) and $TLR4^{KO}$ mouse brains were harvested and homogenised in a highly precise manner using the gentleMACS™ Octo Dissociator. Homogenates were diluted to 10 mg/mL and $^{[3H]}$(-)naloxone added to 1.63 nM. One mL aliquots were vacuum filtered and washed with 5 mL of 0.9% PBS at required time points. $^{[3H]}$(-)Naloxone dissociation was initiated by adding 1 µM of cold drug. Data were analysed using non-linear regressions. Our pilot data has demonstrated a significant change in (-)naloxone binding association kinetics between WT mouse brain homogenates ($2.28x10^{-9}$ sec n=6) and $TLR4^{KO}$ homogenates ($1.33x10^{-9}$ sec n=6). This was represented by faster (-)-naloxone association in the genetic absence of TLR4 compared to wildtype conditions ($P<0.0005$).

However, unexpectedly we did not observe a change (P>0.5) in total $^{[3H]}$(-) -naloxone binding between the WT (12982 DPM +/- 847 n=3) and TLR4^{KO} (13895 DPM +/- 1169 n=3) brain homogenates. We also did not observe any $^{[3H]}$(-)naloxone displacement by classical TLR4 and MD-2 drugs such as LPS or curcumin. These results further suggest an involvement of TLR4 in opioid pharmacology, but the complexity underlying this TLR4 opioid interaction is still enigmatic.

Disclosures: J. Thomas: None. **A.A. Somogyi:** None. **K.C. Rice:** None. **M.R. Hutchinson:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH (1U01 NS066911-01A1; HSW)

Title: Characterization of Galanin receptor 1 knock-out mice in animal models of seizures

Authors: *S. A. GAGANGRAS[1], C. METCALF[2], E. DAHLE[1], B. KLEIN[1,2], H. WHITE[1,2]; [1]Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT; [2]NeuroAdjuvants, Inc., Salt Lake City, UT

Abstract: Galanin is known to play a crucial role in the control of neuronal excitability. However, the contribution of each of the 3 identified galanin receptors is not known. Transgenic animals have been useful in studying the discrete role of each galanin receptor subtype. Previously, it was reported that galanin overexpressing mice show delayed kindling, suggesting the potential role of galanin in epileptogenesis. In the present study, Galanin receptor 1 knock-out C57BL/6J male mice were evaluated for their kindling acquisition rate and we report that the protection in corneal kindling model is afforded by GalR1. GalR1 KO animals show lower threshold to electroconvulsive stimuli in the initial phase of kindling and attain stage 5 generalized seizures in 7.25 ± 1.05 stimulations relative to 12.44 ± 1.76 stimulations in WT ($p<0.05$). However, the total number of stimulations required to acquire the fully kindled state was not different between groups. Furthermore, no significant differences were observed in the seizure thresholds of WT and GalR1 KO mice in 6 Hz psychomotor seizure test. Using quantitative RTPCR analysis from hippocampal tissue of 5 week old male mice, we report that GalR1 KO mice display a ~2-fold increase in the expression of GalR3 (1.78 ± 0.05, $P<0.05$)

relative to WT animals without any significant changes in GalR2 expression. These preliminary results suggest that GalR1 plays a crucial role in network excitability of the corneal kindled mouse and that disruption of GalR1 results in compensatory change in expression of GalR3. Possible implications of GalR3 upregulation in GalR1 KO mice are being currently investigated in different behavioral models.

Disclosures: S.A. Gagangras: None. **C. Metcalf:** A. Employment/Salary (full or part-time):; NeuroAdjuvants, Inc.,Salt lake city, UT, USA. **E. Dahle:** None. **B. Klein:** A. Employment/Salary (full or part-time):; NeuroAdjuvants, Inc.,Salt lake city, UT, USA. **H. White:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroAdjuvants, Inc.,Salt lake city, UT, USA.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

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Program#/Poster#: 223.15/C10

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant HL 098589

Title: Galanin localization and actions in the guinea pig cardiac plexus

Authors: *J. C. HARDWICK[1], A. A. LINTON[2], E. N. POWERS[3];
[1]Ithaca Col., ITHACA, NY; [3]Biol., [2]Ithaca Col., Ithaca, NY

Abstract: Peptidergic modulation of autonomic neurons is an important factor in regulating overall autonomic output. Galanin has been shown to modulate parasympathetic neuronal activity in several vertebrate systems, including the cardiac control pathways. In the current study, we examined the localization and function of galanin in the guinea pig cardiac plexus. Galanin immunreactivity was observed in cell bodies and fibers within the ganglia using an antibody against the human form of galanin. The galanin-immunoreactivity showed no co-localization with the sensory peptide, substance P, but did show some co-labeling with choline acetyltransferase. Using intracellular voltage recordings from whole mount preparations of the guinea pig cardiac plexus, we examined the modulatory effects of galanin on neuronal properties. Direct application of galanin (10-4M) by local pressure ejection, or by inclusion in the bath solution (10-7M) produced a depolarization of the membrane potential and a decrease in input resistance. Some cells also demonstrated an increase in neuronal excitability, as seen as a

decrease in rheobase and/or an increase in the number of evoked action potentials with depolarizing current steps. These results indicate that galanin is found within the cardiac plexus and the peptide can directly affect intracardiac neurons. Thus, galanin may be an important modulator of neuronal function and overall parasympathetic output.

Disclosures: J.C. Hardwick: None. **A.A. Linton:** None. **E.N. Powers:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

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Topic: B.01. Neurotransmitters and Signaling Molecules

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CONICYT-PFB 12/2007 to NCI

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Title: Wnt5a regulates expression of ROCK2 and SYNGAP1 through Mir-101b in rat hippocampal neurons

Authors: *J. CODOCEDO, N. C. INESTROSA;
Pontificia Univ. Catolica De Chile, Santiago, Chile

Abstract: In rat hippocampal neurons, Wnt5a stimulation generates an increase in the clustering of PSD-95 as well as an increase in the density of dendritic protrusions. Additionally, experiments in rat hippocampal slices showed that Wnt5a modulates synaptic activity increasing long-term potentiation.
On the other hand, microRNAs (miRNAs) are a family of endogenously small non-coding RNAs, which control gene expression of their mRNAs targets by hybridization with complementary sequences in the 3' UTR inhibiting its translation. Emerging evidence indicates that the miRNAs are actively involved in the regulation of synaptic plasticity processes mediated by substances such as serotonin, glutamate and BDNF.
Considering that in many models of synaptic plasticity, it has been described that remodeling processes of dendritic spines are dependent on protein translation, we propose that the effects of Wnt5a, are mediated by the action of miRNAs which in turn control the translation of synaptic proteins.

By PCR arrays, we determined the effect of Wnt5a on the expression of 263 miRNAs in rat hippocampal neurons. Using bioinformatics, we determined the predicted mRNA targets as well as their potential role on different signaling pathways. By western blot and inmunofluorescence analysis, we determined the expression change of different targets in neurons treated with Wnt5a. 31 miRNAs decreased and 3 miRNAs increased their expression levels in the presence of Wnt5a. One of the miRNAs that showed a robust decrease in the presence of Wnt5a is miR-101b.
The expression levels of 3 predicted targets of miR-101b, ROCK2, SYNGAP1 and COX-2, showed a significant increase in their expression levels in the presence of Wnt5a.This is the first report showing that Wnt5a is able to regulate the levels of miRNAs in hippocampal neurons. Interestingly, the signaling pathways regulated by the predicted targets correspond to processes in which Wnt5a has a key role, including cancer and regulation of the actin cytoskeleton. The regulation through miRNAs, of targets like ROCK2 and SYNGAP1 can help to understand the role of Wnt5a in the synaptic modulation.

Disclosures: J. Codocedo: None. **N.C. Inestrosa:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant P30DA018310

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Title: D-amino acid-containing peptides in the nervous systems of vertebrate and invertebrate models

Authors: LIVNAT[1], H.-C. TAI[2], S. RUBAKHIN[2], J. V. SWEEDLER[2];
[1]Chem., Univ. of Illinois At Urbana-Champaign, Urbana, IL; [2]Univ. of Illinois in Urbana-Champaign, Urbana, IL

Abstract: Many neuropeptides are post-translationally modified in the process of producing the final, bioactive peptide; these modifications can be difficult to predict from sequence information alone, requiring their measurement. Peptide isomerization is a rare post-translational modification (PTM) where one residue of a peptide is enzymatically converted from an L-amino

acid to a D-amino acid, resulting in a D-amino acid-containing peptide (DAACP). Isomerization usually occurs near the C- or N-terminus and produces significant changes in the peptide's structure and bioactivity, as well as increases its resistance to degradation by peptidases. Only a small number of DAACPs have been discovered. Our hypothesis is that there are a small number of known DAACPs, at least in part, because the L- to D- conversion does not alter a peptide's mass, and mass spectrometry has become the method used to characterize neuropeptides. To address this issue, we created an alternative strategy to discover new DAACPs that take advantage of the properties conferred by isomerization. Briefly, potential DAACPs are determined by their resistance to degradation by a leucine aminopeptidase, aminopeptidase M (APM) or by homology of a peptide's sequence to known DAACPs. Potential DAACPs are purified using liquid chromatography and then acid hydrolyzed into their component amino acids. The chirality of these amino acids is assayed by derivatization with Marfey's reagent (1-Fluoro-2,4-dinitrophenyl-5-L-alanine amide, FDAA) and determined using LC coupled to a triple-quadrupole mass spectrometer. These techniques are being applied to the central nervous system of the mollusk Aplysia californica. We have already identified a novel DAACP, GdFFD, where the dF indicates that isomerization occurs at the second residue from the N-terminus. For GdFFD, the all L-peptide is much less active on the feeding network than the DAACP. In addition, other putative DAACPs in A. californica have been observed, such as LAARLI, a peptide from the procerebrin prohormone where isomerization appears to occur at the second residue from the N-terminus, an alanine. Importantly, the advantage of the described approach is that it is not limited to a particular model system and can be applied to determination of putative DAACPs from the mammalian nervous system.

Disclosures: Livnat: None. **H. Tai:** None. **S. Rubakhin:** None. **J.V. Sweedler:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIEHS Grant ES090082-15

Title: Endogenous substance p regulates microglial density in substantia nigra through neurokinin-1 receptor-nadph oxidase axis-mediated chemotaxis

Authors: *Q. WANG[1], C.-H. CHU[2], S.-H. CHEN[2], B. WILSON[2], J.-S. HONG[2];
[1]Neuropharm. Section, NIEHS, RTP, NC; [2]NIEHS, RTP, NC

Abstract: The distribution and density of microglia differ greatly among brain regions. We have previously reported the density of microglia in the substantia nigra (SN) is 5 fold higher than other brain regions, implicating why the SN is selectively more sensitive to the neuroinflammation-mediated pathogenesis of Parkinson's disease (PD). However, the reason for the high density of nigral microglia is not known. We hypothesized that substance P (SP), a major endogenous pro-inflammatory peptide stored at high concentrations in the SN, is a major regulator for the high density of nigral microglia. Developmental studies revealed that nigral microglial density peaked around postnatal 30 (P30). In contrast, SP was detected at high levels in SN as early as P1. Transgenic mice incapable of producing SP (*TAC1*$^{-/-}$) exhibited reduced nigral microglial density compared to wild type (WT) controls. This finding led us to speculate that SP may attract the migration of microglia toward to the SN. We confirmed the chemotactic property of SP *in vitro* by demonstrating that SP induced the migration of microglia in a transwell culture system. *In vivo* studies further showed facilitated directional migration of transplanted enhance-green fluorescent protein (EGFP)-labeled microglia towards the brain region injected with SP in *TAC1*$^{-/-}$ mice. Additional studies on the signaling pathways mediating chemotaxis by SP revealed that both neurokinin-1 receptor (NK1R), the G-protein coupled receptor for SP, and NADPH oxidase (NOX2, a key superoxide-producing enzyme on microglia) are necessary for the chemoattractant properties of SP. Results showed that SP-induced migration of microglia prepared from either NK1R$^{-/-}$ or NOX2$^{-/-}$ mice was greatly reduced. Furthermore, pharmacological inhibition of NK1R or NOX2 showed a similar inhibition. Evidence suggesting a cross talk between NK1R and NOX2 was observed by a finding showing SP-stimulated NOX2 activation, as measured by membrane translocation of p47^{phox} (a cytosolic subunit of NOX2) and subsequent release of superoxide were mediated through NK1R/β-arrestin1-dependent pathways. In summary, these results strongly suggest that SP is capable of recruiting microglia to the SN through a novel NK1R-NOX2 axis-mediated pathway, accounting for the high microglial density in the SN.

Disclosures: Q. Wang: None. **C. Chu:** None. **S. Chen:** None. **B. Wilson:** None. **J. Hong:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

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Support: AA020023

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AA019767

AA11605

AA007573

Title: HMGB1 nucleocytoplasmic mobilization and release from neurons through decreased HDAC activity

Authors: *J. Y. ZOU, F. T. CREWS;
Univ. North Carolina, Chapel Hill, CHAPEL HILL, NC

Abstract: High-mobility group box 1 (HMGB1) is a ubiquitous nuclear protein that functions as an endogenous danger signaling molecule upon release into the extracellular space during sterile inflammation and infection. HMGB1 release and inflammatory activity in brain remain elusive. The present study uses an *ex vivo* model of organotypic hippocampal-entorhinal cortex (HEC) slice cultures to investigate HMGB1 mobilization and its role in mediating inflammatory activation in brain. Stimulation of HEC slices with various reagents including lipopolysaccharide (LPS), cytokine TNFα, ethanol, glutamate/NMDA and mGluR agonists as well as HDAC inhibitors triggers robust releases of HMGB1 from neurons into culture media. Active secretion (without causing cell death) of neuronal HMGB1 is associated with movement of HDAC1/4 and HMGB1 proteins from nuclear to cytosolic fractions. Co-immunoprecipation analysis indicates that actively secreted neuronal HMGB1 is associated with increased acetylation of HMGB1. We further examined HMGB1-mediated neuroimmune activation in response to LPS and ethanol. Induction of proinflammatory molecules such as TNFα and IL-1β by LPS and ethanol was blunt by siRNAs to HMGB1 and TLR4, HMGB1 neutralizing antibody, HMGB1 inhibitor glycyrrhizin as well as TLR4 antagonist naltrexone. Blockade of microglial activation with minocycline also blunt expression of proinflammatory cytokines induced by ethanol and LPS, suggesting microglial TLR4 may initiate HMGB1-mediated neuroimmune activation in brain. On the other hand, passively released HMGB1 from damaged neurons induced by higher concentrations of glutamate/NMDA was correlated with extend of neuronal cell death. HMGB1 is depleted in nuclei of damaged neurons but uniquely accumulated in neuronal cytoplasm and processes. By using conditioned medium (CM) generated from NMDA-stimulated slice cultures, which contains HMGB1 level about 11ng/ml, we demonstrated that HMGB1 in CM can exert paracrine signaling roles in sensitizing neuronal cells more vulnerable to glutamate/NMDA-induced excitotoxicity, inducing neuroimmune activation and expression of proinflammatory

molecular genes such as TNFα, IL-1β and MMP-9, and stimulating proliferation of microglial progenitors; all danger molecule-like effects of HMGB1 were reduced by HMGB1 neutralizing antibody and/or HMGB1 inhibitor glycyrrhizin. These novel findings underlie the key role of danger signal HMGB1 released from neurons in mediating neuron-glia neuroimmune communication. Our results support the hypothesis that HDACs modulate HMGB1 release and brain neuroimmune activation.

Disclosures: J.Y. Zou: None. **F.T. Crews:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.20/C15

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: FAPERJ

CNPq

PRONEX-MCT

Title: Cytokines regulate photoreceptor differentiation and survival *In vitro*

Authors: *A. SHOLL-FRANCO, A. S. AGOSTINI;
Inst. Biofisica - IBCCF, Univ. Fed Rio De Janeiro, Rio De Janeiro, Brazil

Abstract: Interleukins (ILs) are important members of a class of cytokines that regulate several body functions. In this way, IL-4 and IL-10 were characterized as prototypical anti-inflammatory cytokines with neuromodulatory effects. They have been implicated with neural cell differentiation and survival, as well in inflammatory responses within nervous system. In this work we investigated the effects of IL-4 and IL-10 upon rod photoreceptor differentiation and survival in the developing mouse and rat retina, using monolayer and organotypic cultures to study putative regulatory effects of these cytokines on the development and survival of photoreceptors cells. Cultures were prepared using postnatal day 0 (P0) C57bl/6 mouse or postnatal day 7 (P7) hooded rat retinas maintained for 1, 2, or 3 days in vitro (DIV). For the in vivo studies, we used retinas from P0 to P18 animals. The expression of rhodopsin, IL-4 and IL-10 was analyzed by western blotting and immunocytochemistry using specifics antibodies (polyclonal antibodies against IL-4 and IL-10 - Santa Cruz; monoclonal antibody Rho4D2

against rhodopsin, kindly provided by Dr. R. Molday). On the in vivo retina, rod photoreceptors begin to appear at P0, and in both cultures systems, Rho4D2-positive cells were identified after 1 DIV in a pattern similar to that observed in vivo. IL-4 is expressed both in neural retina and non-neural ocular tissue, while IL-10 was found mainly in non-neural tissue. Our results demonstrated that both cytokines promoted a dose-dependent stimulatory effect, increasing the number of Rho4D2-positive cells (165% and 150%, respectively after 3 DIV with IL-10 or IL-4 treatment). Pretreatment with IL-10 do not prevent cobalt chloride induced specific rod photoreceptor cell death. However, IL-4 pretreatment prevent rod photoreceptor cell death in P7 rat retina in a dose- and time-dependent manner. Blocking antibodies against IL-4 inhibit the protective effect of IL-4, as well as the co-treatment with PKC and tyrosine kinases inhibitors. Overall, these results demonstrate that IL-4 and IL-10 can influence rod photoreceptor differentiation, but only IL-4 block rod photoreceptor cell death induced by cobalt chloride. Moreover, this survival effect is highly specific and is mediated through PKC and tyriosine kinase signaling activation.

Disclosures: A. Sholl-Franco: None. **A.S. Agostini:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.21/C16

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: IL-13 dynamically alters the excitability of dopamine neurons in the ventral tegmental area in wild type but not IL-13Rα1 knockout mice

Authors: M. A. HERMAN[1], *M. ROBERTO[1], B. CONTI[2];
[1]CNAD, [2]Chem. Physiol., Scripps Res. Inst., LA JOLLA, CA

Abstract: The dopamine (DA) system in the ventral tegmental area (VTA) is critically involved in regulating motivation and reinforcing behaviors. Alterations in DA neuron firing have been implicated in a number of clinical disorders including schizophrenia, addiction and attention deficit hyperactivity disorder. The pro-inflammatory cytokine IL-13 binds IL-13 Rα1 which forms a heterodimer with IL-4Rα and initiates a signal cascade involving Stat6 and JAK phosphorylation. IL-13 Rα1 is abundantly expressed in DA neurons in the VTA, however no studies have examined the role of IL-13 in overall activity of DA neurons. In the present study we performed whole cell current-clamp recordings of DA neurons and applied IL-13 to

determine if acute application produced any measurable effects on DA neuron firing in wild-type (WT) and IL-13 Rα1 knockout (IL-13 Ra KO) mice. DA neurons were identified by previously described membrane properties and action potential characteristics. Acute application of IL-13 (10 ng/ml) to DA VTA neurons from WT mice significantly decreased the resting membrane potential from -46.3 ± 1.1 mV to -54.6 ± 2.1 mV and significantly decreased firing from 15.5 ±2.8 Hz to 3.9 ± 3.3 Hz and in some cases inhibited firing altogether ($p<0.05$ by paired t-test; n = 9). The hyperpolarization and decrease in firing with IL-13 application displayed an almost immediate onset and was reversible upon washout. Whole cell current-clamp recordings were also performed in VTA DA neurons from IL-13 Rα1 KO mice, which displayed no significant change in resting membrane potential but a significantly lower baseline firing rate as compared to WT mice (9.7 ± 1.4 Hz as compared to 18.4 ± 3.0 Hz); $p<0.05$ by unpaired t-test; n = 29 and 17, respectively). In addition, acute application of IL-13 to IL-13 Ra KO mice did not significantly alter resting membrane potential or firing rate. Collectively these data demonstrate that IL-13 can dynamically and reversibly alter DA neuron activity through actions at IL-13 Rα1 in the VTA, suggesting a more immediate intersection of immune and/or stress responsivity and neural excitability in the VTA.

Disclosures: M.A. Herman: None. **M. Roberto:** None. **B. Conti:** None.

Poster

223. **"Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.22/C17

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Il-1β and Il-1RA release in the brain as measured by *In vivo* microdialysis and its dependence on P2X7 receptor activation

Authors: D. SONG[1], H. ZHOU[1], A. T. HOPPER[2], *G. N. SMAGIN[1];
[1]Neuroinflam. DBU, [2]Medicinal Chem., Lundbeck, Paramus, NJ

Abstract: Neuroinflammation (NI) is a central component of several neurological disorders and is primarily mediated by microglia, the myeloid cell population of the CNS. IL-1β is a central mediator of NI, which is released from activated microglia. This process involves a priming step to induce pro-IL-1β and a triggering event leading to the processing and release of mature IL-1β. This “double hit” paradigm is experimentally mimicked by priming with lipopolysaccharide (LPS) and activation of the P2X7 receptor with an agonist such as BzATP. IL-1 receptor

antagonist (IL-1RA) is a member of the IL-1 cytokine family. IL-1RA is secreted by various types of cells including immune cells, and is an endogenous inhibitor of the pro-inflammatory effect of IL-1β. This protein inhibits the activities of ILs 1α and β and modulates a variety of IL-1 related inflammatory responses. In this study we investigated the BzATP-stimulated release of IL-1β and IL-1RA in the CNS by microdialysis and their dependence on P2X7 receptor activation.
Rats were implanted with the guide cannula placed into the prefrontal cortex (PFC). An additional cannula (22 ga, 4.5 mm long) for i.c.v. injections was implanted into the lateral cerebral ventricle. One week after surgery, a microdialysis probe (4 mm active membrane, 3000 kDa MWKO) was implanted. The LPS and P2X7 agonist BzATP alone or in a "double hit" paradigm were administered i.c.v. A selective P2X7 antagonist was systemically administered one hour prior to BzATP. Microdialysis samples were analyzed for IL-1β and IL-1RA by an MSD platform assay.
LPS (3 µg) or BzATP (50 µg) alone did not affect IL-1β or IL-1RA concentrations in microdialysates, with the stable basal levels at 12 and 310 pg/ml, respectively. Administration of BzATP (87 µg) 1 hour after LPS ("double hit") increased IL-1β and IL-1RA concentrations in microdialysates collected from the PFC to 250 and 1000 pg/ml respectively, with the maximum at 2 h after BzATP administration. Administration of the P2X7 antagonist attenuated the LPS/BzATP-induced increase of IL-1β and IL-1RA.
We demonstrate that an improved collection method enables the study of changes of cytokine concentrations in the brain. The results demonstrate that after priming with LPS, the release of IL-1β in the brain is stimulated by a P2X7 agonist and attenuated by a P2X7 antagonist in vivo. Increase in IL-1RA concentrations in the microdialysates occurs approximately at the same time as IL-1β. We show that a brain-penetrant P2X7 antagonist can reduce CNS IL-1β and IL-1RA concentrations in extracellular fluid and might provide an entry point for therapeutic intervention.

Disclosures: D. Song: A. Employment/Salary (full or part-time):; Lundbeck. **H. Zhou:** A. Employment/Salary (full or part-time):; Lundbeck. **G.N. Smagin:** A. Employment/Salary (full or part-time):; Lundbeck. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lundbeck. **A.T. Hopper:** A. Employment/Salary (full or part-time):; Lundbeck.

Poster

223. "Cytokines and Invertebrate Neurotransmitters: Expression, Regulation, and Function"

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 223.23/C18

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Regione Autonoma della Sardegna-CRP10810

Title: Interferon-β alters neurotrophin-3 TrkC receptor expression and signaling in differentiated human SH-SY5Y neuroblastoma cells

Authors: S. DEDONI, M. C. OLIANAS, *P. ONALI;
Univ. of Cagliari, Monserrato (Ca), Italy

Abstract: Both type I interferons (IFNs) and neurotrophins act on neuronal cells to regulate survival, growth and differentiation. However, relatively little is known on the interaction between these two classes of regulatory proteins. We have previously reported that IFN-β causes down-regulation of the brain-derived neurotrophic factor/TrkB receptor function and up-regulation of the nerve growth factor/TrkA/p75 receptor complex. In the present study, we investigated the effects of long-term exposure to IFN-β on the functional activity of the neurotrophin 3 (NT3)/TrkC receptor system in differentiated SH-SY5Y human neuroblastoma cells. We found that IFN-β treatment curtailed NT3-induced activation of distinct signaling molecules regulated by TrkC receptor, including protein kinase B/Akt, phospholipase Cγ1 and extracellular signal-regulated kinase 1 and 2. Analysis of TrkC receptor expression showed the presence of both 140 kDa full-length and 90-100 kDa kinase-deficient truncated isoforms. Exposure to IFN-β induced a predominant enhancement in the levels of the truncated TrkC receptor isoform in a time-dependent fashion. As the main function of the truncated receptor is the inhibition of the kinase-active TrkC receptor isoforms, our data suggest that long-term exposure to IFN-β down-regulates NT3 signaling by altering the balance between kinase-active and inactive TrkC receptor isoforms.

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

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.01/C19

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant ES019282

Title: Tonic activation of α7 nicotinic acetylcholine receptors (nAChRs) controls glutamatergic inputs to striatal and parietal cortical neurons in guinea pig brain slices

Authors: *E. X. ALBUQUERQUE, E. F. R. PEREIRA, M. ALKONDON;
Div. of Translational Toxicology, Dept Epidemiology and Publ. Hlth., Univ. Maryland Sch. Med., Baltimore, MD

Abstract: Recent studies from our laboratory have shown that in rat hippocampal slices tonically active α7 nAChRs modulate spontaneous GABAergic and glutamatergic synaptic transmission in CA1 pyramidal neurons (JPET 341: 500, 2012; Biochem Pharmacol 84: 1078, 2012). The present study was designed to test the hypothesis that this mechanism is conserved in brain regions known to be sensitive to the toxic effects of irreversible inhibition of acetylcholinesterase, the enzyme that hydrolyzes acetylcholine. To this end, whole-cell patch clamp recordings were obtained at -60 mV from various neurons of guinea pig striatal and parietal cortical (PC) slices from 32-52 day-old female guinea pigs. Recordings were done in ACSF containing the muscarinic receptor antagonist atropine (0.5 μM) and the $GABA_A$ receptor antagonist picrotoxin (50 μM). Biocytin was included in the pipette solution, and at the end of the recording, slices were processed and the neuron types identified. A 15-min bath application of α7 nAChR antagonist methyllycaconitine (10 nM) decreased the frequency of excitatory postsynaptic currents (EPSCs) without changing the mean peak amplitude, 10-90% rise time, and decay time constants of these currents. The reduction in the frequency of EPSCs was observed in two out of three striatal neurons and two out of four PC neurons. Biocytin images revealed that the studied neurons were the pyramidal type located in layers III and IV of the PC, and medium spiny neurons in the striatum. These results support the hypothesis that tonically active α7 nAChRs control glutamate transmission in the PC and striatum of guinea pigs.

Disclosures: E.X. Albuquerque: None. **E.F.R. Pereira:** None. **M. Alkondon:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.02/C20

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant NS25296

Title: Presence of non-α7 nAChRs in CA1 pyramidal neurons in rat hippocampal slices

Authors: *M. ALKONDON, E. F. R. PEREIRA, E. X. ALBUQUERQUE;
Div. of Translational Toxicology, Dept. Epidemiology and Publ. Hlth., Univ. Maryland Sch. Med., Baltimore, MD

Abstract: Electrophysiological studies from several laboratories have confirmed the presence of both α7 (methyllycaconitine (MLA)-sensitive) and non-α7 (dihydro-β-erythroidine-sensitive) functional nAChRs in various interneurons in hippocampal slices. Few studies have shown the presence of α7 nAChRs in CA3 and CA1 pyramidal neurons. Tonic activation of these α7 nAChRs maintains the frequency of spontaneous glutamate excitatory postsynaptic currents (EPSCs) in CA1 pyramidal neurons. Further, activation of mecamylamine (MEC)-sensitive non-α7 nAChRs by basal levels of acetylcholine in hippocampal slices or by exogenously applied agonists results in glutamate release onto stratum radiatum interneurons. However, the location of MEC-sensitive nAChRs and its influence on other pyramidal neurons have never been studied. To address this issue, we obtained whole-cell patch clamp recordings from CA1 pyramidal neurons in rat hippocampal slices. In the presence of the muscarinic receptor antagonist atropine (0.5 μM), the $GABA_A$ receptor antagonist bicuculline (10 μM), and the α7 nAChR antagonist MLA (3-10 nM), U-tube application of acetylcholine (0.1 mM) induced inward currents at -60 mV. These currents persisted in presence of NMDA receptor antagonist APV (50 μM), but were suppressed in presence of 3 μM MEC. On the other hand, U-tube application of acetylcholine (0.1 mM) to CA1 pyramidal neurons held at +40 mV induced outward-going EPSCs that were suppressed by APV (50 μM), kynurenic acid (100-200 μM) or MEC (3 μM). These results support the notion that MEC-sensitive non-α7 nAChRs are located on somatodendritic regions of CA1 pyramidal neurons and their activation results in glutamate release on to neighboring pyramidal neurons.

Disclosures: M. Alkondon: None. **E.F.R. Pereira:** None. **E.X. Albuquerque:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.03/C21

Topic: B.02. Ligand Gated Ion Channels

Support: NIEHS/NIH intramural research program

Title: Probing α7 nicotinic acetylcholine receptor-mediated signaling pathways in cultured hippocampal neurons

Authors: *Q. CHENG, J. L. YAKEL;
Lab. of Neurobio., NIEHS, Durham, NC

Abstract: The activation of α7 nicotinic acetylcholine receptors (nAChRs) have been shown to improve hippocampal-dependent learning and memory, and rescue the deficits in mossy fiber

LTP. Therefore, it is important to understand the molecular mechanism of α7 nAChRs' action. Since the α7 nAChRs are highly permeable to calcium, some studies have suggested that activation of nAChRs could activate calcium-dependent signaling molecules, as well as protein kinase A (PKA). Moreover, a recent study showed that the α7 nAChR is associated with adenyl cyclase I (AC1) within lipid rafts. However, the direct link between the activation of the α7 nAChR and cyclic adenosine monophosphate (cAMP) signaling in neurons is still lacking. To address this question, we used a Förster- Resonance Energy Transfer (FRET)-based sensor (mTurquoise-Epac(CD, ΔDEP)-^{cp173}Venus-Venus) to monitor the intracellular level of cAMP in cultured hippocampal neurons. We found that application of the α7 nAChR agonist, PNU 282987, induced a significant increase in intracellular cAMP levels measured with this FRET-based sensor. To determine the requirement for the α7 nAChR, we will compare α7 nAChR agonist-induced cAMP changes between wild type and α7 nAChR knockout neurons. To determine the involvement of AC1, we will test the effect of shRNAi against AC1. Lastly, we will attempt to correlate the changes in cAMP levels with neurotransmitter release via a pH-sensitive reporter protein (synaptophysin-pHTomato). Our findings may yield some insight into the molecular mechanisms of the positive cognitive actions of α7 nAChR agonists, and development of therapeutic treatments for cognition impairments.

Disclosures: Q. Cheng: None. **J.L. Yakel:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.04/C22

Topic: B.02. Ligand Gated Ion Channels

Title: Shedding some light on nAChRs: Discovery of fluorescent α7 agonists and antagonists

Authors: J. BURGI[1], *J. S. SPROUSE[2], S. BERTRAND[3], E. NEVEU[3], T. SCHAER[3], J.-L. REYMOND[1], D. BERTRAND[3];
[1]Dept. of Chem. and Biochem., Univ. of Berne, Berne, Switzerland; [2]Sprouse Consulting LLC, Stonington, CT; [3]HiQScreen, Geneva, Switzerland

Abstract: The introduction of fluorescent tools has provided significant advantages in various fields of biology with the possibility of localizing cells and proteins down to the single molecule level. While fluorescent labeling of nicotinic acetylcholine receptor (nAChR) antagonists has been successfully achieved with, for example, the snake toxin α-bungarotoxin (α-Bgt), attempts to develop fluorescent small molecule agonists have so far been largely unsuccessful.

In this work we have investigated the combination of α7 nAChR-selective chemical scaffolds with fluorophores in an attempt to develop α7 specific agonists that could be used for histological localization of the receptors as well as for electrophysiology. Knowledge gained from virtual and functional screening allowed us to engineer new agonist molecules retaining high affinity for the α7 nAChRs while maintaining the fluorescent properties of the label. Pharmacological characterization of the fluorescent probes was conducted at human nAChRs expressed in *Xenopus* oocytes. Both antagonists and agonists were identified and characterized at different receptor subtypes to demonstrate the selectivity of these probes. Fluorescent measurements were conducted in cells expressing the α7 receptors and under primary culture conditions, and compared with results obtained with fluorescent α-Bgt.
The data generated suggest that the specificity of small molecules can be maintained allowing the design of new tools to investigate the functional properties of nAChRs. Illustrating the importance of careful design in small molecule labeling, these data reveal new strategies that will find important applications in the localization and characterization of receptors as well as in drug design.

Disclosures: J. Burgi: None. **J.S. Sprouse:** F. Consulting Fees (e.g., advisory boards); HiQScreen. **S. Bertrand:** A. Employment/Salary (full or part-time):; HiQScreen. **E. Neveu:** A. Employment/Salary (full or part-time):; HiQScreen. **T. Schaer:** A. Employment/Salary (full or part-time):; HiQScreen. **J. Reymond:** None. **D. Bertrand:** A. Employment/Salary (full or part-time):; HiQScreen.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.05/C23

Topic: B.02. Ligand Gated Ion Channels

Support: NSERC Grant

Canadian Foundation for Innovation Grant

Victoria Foundation

NARSAD

British Columbia Knowledge Development Fund

Title: T cell receptor activation decreases excitability of neocortical interneurons by inhibiting α7 nicotinic receptors

Authors: *P. KOMAL[1], G. GUDAVICIUS[2], C. NELSON[2], R. NASHMI[1];
[1]Dept. of Biol., [2]Dept. of Biochem. and Microbiology, Univ. of Victoria, Victoria, BC, Canada

Abstract: T cell receptors (TCR) are expressed on the surface of T lymphocytes where its role in adaptive immunity is well described. Recently, immune proteins have also been found to be expressed in the brain. One such class of proteins are TCRs, which are expressed in the cerebrel cortex of the CNS, though their function remains largely unknown. α7 nicotinic acetylcholine receptors (nAChRs) are a major class of ligand-gated ion channels in the brain, implicated to play an important role in cognition, attention, working memory and synaptic plasticity. Since α7 receptors are expressed in the same brain region where TCR expression is localized, we investigated whether TCRs may potentially functionally interact with α7 nAChRs. Whole-cell recordings were performed in Jurkat cells transfected with α7 nAChRs and from layer 1 neurons of prefrontal cortical brain slices. Concanavillin A (ConA, TCR agonist) incubation significantly attenuated α7 mediated nicotinic receptor currents both in Jurkat cells and in layer 1 cortical interneurons. Genistein (a tyrosine kinase inhibitor) pre-incubation rescued the effects of ConA on acetylcholine (ACh) mediated responses. We then examined if src family kinases directly phosphorylate the tyrosine (Y442) residue located in the M3-M4 cytoplasmic loop of α7 receptors by using mutant α7(Y442A) nAChRs. ConA stimulation did not have any effect on α7(Y442A) mediated nAChR currents. Immunoprecipitation experiments showed that TCR activation significantly increased tyrosine phosphorylation of α7 nAChRs. To examine the mechanism of action of TCRs, Alexa-647 α-bungarotoxin surface labelling of α7 receptors in Jurkat cells showed that TCR activation reduced α7 receptor surface expression. Furthermore, current fluctuation analysis revealed that TCR stimulation decreased single-channel conductance of α7 nAChRs without altering gating kinetics. Current-clamp recordings on layer 1 cortical interneurons showed that TCR activation with ConA or α7 nAChR inhibition with the antagonist MLA both reduced the excitability of layer 1 neurons of the cortex. Our study provides evidence that TCRs do have a neuronal function in the CNS. Activation of TCRs attenuate neuronal excitability by negatively modulating α7 nAChR activity.

Disclosures: P. Komal: None. **G. Gudavicius:** None. **C. Nelson:** None. **R. Nashmi:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.06/C24

Topic: B.02. Ligand Gated Ion Channels

Support: NIH grant GM57481

Asmacure Ltée research grant

Title: A minimal pharmacophore for a nicotinic alpha7 silent agonist, relevance for the therapeutic development of treatments for asthma

Authors: *C. STOKES[1], E. ISRAËL-ASSAYAG[2], R. L. PAPKE[1];
[1]Pharmacol & Therapeut., Univ. Florida, GAINESVILLE, FL; [2]Asmacure Ltée, Quebec City, QC, Canada

Abstract: ASM-024 (1,1 di-ethyl-4-phenylhomopiperazinium), a compound in Phase 2 trials for the treatment of asthma and COPD, has been shown to be active at inhibiting methacholine as well as histamine-induced contraction of tracheal and bronchial smooth muscle and at inhibiting the LPS-evoked release of proinflammatory cytokines by cultured monocytes. We demonstrated that ASM-024 decreases muscarinic responses to acetylcholine of M1, M2, and M3 mAChR expressed in *Xenopus* oocytes. Due to the structural relationship between ASM-024 and the ganglionic nAChR agonist 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP), we evaluated the effects of ASM-024 on human nAChR subtypes expressed in *Xenopus* oocytes. ASM-024 inhibited the ACh-evoked responses of α3β4 and α7 receptors in co-application experiments. Competition studies indicated that antagonism of α3β4 was noncompetitive while a significant component of the α7 antagonism was competitive. A new type of α7 ligand has recently come to light: molecules which on their own produce little or no channel activation but effectively activate the ion channel when combined with a Type II positive allosteric modulator (PAM). Such "silent agonists" have been implicated as potential mediators of α7-dependent anti-inflammatory effects, and we confirmed that ASM-024 is an α7 silent agonist, since it activated α7 ion channels when co-applied with PNU-120596. DMPP is an efficacious agonist of α7, and one of the structural features distinguishing DMPP from ASM-024 is the presence of additional methyl groups on the quaternary ammonium. While tetramethylammonium is an efficacious α7 agonist, tetraethylammonium (TEA) is a competitive antagonist of α7 ACh-evoked responses. TEA was also confirmed to be a silent agonist, since it produced ion-channel activation when co-applied with PNU-120596. Triethylmethylammonium produced direct α7 channel activation, while TEA does not, defining TEA as the minimal structure required for PAM-dependent α7 activation. ASM-024 shows that the rudimentary silent agonist element of TEA may be incorporated in other more complex structures, suggesting that the TEA portion of ASM-024 prevents activation of ganglionic nAChR while permitting activation of α7-mediated anti-inflammatory pathways.

Disclosures: C. Stokes: None. **E. Israël-Assayag:** A. Employment/Salary (full or part-time):; Asmacure Ltée. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Asmacure Ltée. **R.L. Papke:**

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.; Asmacure Ltée.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.07/C25

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant GM57481

NIH Grant DA027113

SK Biopharmaceuticals research award

Title: Mutations of alpha7 nAChR W55 enhance the allosteric agonism of GAT107, the active isomer of 4BP-TQS, and decouple interactions between orthosteric and allosteric sites

Authors: *R. L. PAPKE[1], N. A. HORENSTEIN[2], C. STOKES[3], C.-Y. MAENG[4], A. R. KULKARNI[5], G. A. THAKUR[6];
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Abstract: GAT107, the (+)-enantiomer of racemic 4BP-TQS with 3aR, 4S, 9bS absolute stereochemistry, is a strong positive allosteric modulator (PAM) of α7 nAChR with intrinsic agonist (IA) activity, while GAT106, the (-)- enantiomer with 3aS, 4R, 9bR stereochemistry, is inactive and does not compete with GAT107. There are two fundamental modes for the positive allosteric modulation of α7 nAChR: one mode, barrier modulation (BM), which affects the energy barriers between conducting and nonconducting states but not absolute free energy of the states, will operate on a population of receptors responding synchronously to agonist application to produce a transient increase in channel opening. The other mode, equilibrium modulation (EM), which affects the relative stability of conducting and nonconducting (i.e. desensitized) states, will produce protracted increases in current and may reverse some forms desensitization (Ds states) induced by agonist, while other nonconducting states are insensitive (Di states). Like PNU-120596, GAT107 has both BM and EM effects. The IA activity of racemic 4BP-TQS was hypothesized to be due solely to binding at the same allosteric site. However, it is unclear if this is the case since the IA effects are only manifested when GAT107 is in the external solution,

while the PAM effects persist long after the free drug is washed away. Prolonged application of GAT107 alone produced primarily IA and EM effects with protracted activation. Addition of the orthosteric agonist (OA) ACh resulted in transient activation followed by PAM-insensitive desensitization. We have previously characterized W55 as a fulcrum within the extended orthosteric ligand binding domain such that mutations of this residue have varying effects depending on specific ligands and receptor subunit composition. We determined that a range of amino acid substitutions at W55 in α7 had the common effect of increasing the IA activity of GAT107 and imbuing the PAMs TQS and PNU-12596 with IA activity. For the W55A mutant the IA activity was decoupled from the effects of OAs. The IA effects of PNU-120596 were additive with OAs, while for TQS OA effects depended on the PAM concentration. While the IA effects of GAT107 in the wild-type α7 nAChR were sensitive to the orthosteric antagonist/inverse agonist MLA, the IA activity of GAT107 for the W55A mutant was MLA insensitive. Our data suggest that a further fulcrum effect of W55 is a containment/limitation of positive modulation produced by allosteric ligands. Homology modeling suggests that Y93 on the primary face of the LBD may be a partner to W55 on the complementary side in providing this containment, a hypothesis we have confirmed with mutation of Y93.

Disclosures: R.L. Papke: None. **N.A. Horenstein:** None. **C. Stokes:** None. **C. Maeng:** None. **A.R. Kulkarni:** None. **G.A. Thakur:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.08/C26

Topic: B.02. Ligand Gated Ion Channels

Support: NIH grant GM57481

Title: Silent agonists of alpha7 nicotinic acetylcholine receptors require positive allosteric modulators for channel activation

Authors: *K. CHOJNACKA[1], R. L. PAPKE[2], N. A. HORENSTEIN[1];
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Abstract: The α7 nicotinic acetylcholine receptor (nAChR) is a homopentameric ligand-gated ion channel that is considered to play an important role in cognition, sensory gating, and neuroprotection in the CNS. There is also growing appreciation that α7 nAChRs are able to modulate inflammatory responses mediated by non-neuronal cells and do so in a way that may not involve ionotropic behavior. There are at least two different desensitized states of α7 nAChR:

D_s, which is sensitive to type II positive allosteric modulators (PAMs) such as PNU-120596, and D_i, which is insensitive to type II PAMs. We introduce new non-traditional ligands, "silent agonists", that bind to the conventional acetylcholine binding site with little or no probability of ion channel activation, but that place the receptor selectively into the D_s state. We have classified three structurally unique groups of molecules that act as silent agonists. One group is exemplified by benzylidene anabaseine-type molecules such as 3-pyridinylmethylene anabaseine (3PAB). The second group features compounds that contain a positively charged ring, a central ring with hydrogen bonding capability, and a flanking aryl group. The third group is represented by bulky quaternary alkyl ammonium compounds (see Stokes et al. presentation this session). To explore the molecular features of PAM-dependent activators from group 2, we designed and synthesized a series of compounds (KC-1 to KC-9). 5'-Phenylanabaseine (KC-1) was made in three steps by Suzuki coupling and organolithium addition to N-Boc-2-piperidinone, and we identified it as a new silent agonist. KC-1 showed essentially no conventional agonism when tested at 100 μM on human α7 nAChR expressed in *Xenopus* oocytes (net charge 0.083 ± 0.009 of ACh control responses), it reduced responses of α7 to acetylcholine (IC_{50} = 41 ± 5 μM), and when co-applied at 100 μM with type II PAMs, it produced greatly potentiated currents when compared to 60 μM control ACh applications (21-fold higher with 10 μM PNU-120596 and 4-fold higher with 30 μM TQS). The data indicate that KC-1 is likely to bind to the same site as traditional α7 agonists because 100 nM of the nAChR competitive antagonist MLA was 100 % effective at blocking responses to 100 μM KC-1 co-applied with 10 μM PNU-120596. Two of the compounds in the series of close KC-1 analogs (KC-5 and KC-7) were also identified as new silent agonists. Type II PAM–dependent activators of α7 may find use as tools to study functions of the receptor that do not involve ion channel opening and may also constitute a new class of α7 therapeutics.

Disclosures: K. Chojnacka: None. **R.L. Papke:** None. **N.A. Horenstein:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.09/C27

Topic: B.02. Ligand Gated Ion Channels

Title: Effects of positive allosteric modulators (PAMs) of nicotinic receptors on intracellular Ca2+ concentration [Ca2+]i in mouse embryonic mesencephalic neurons in primary culture

Authors: *C. COSI[1], F. MARTY[1], P. MAILLOS[2], P. C. MOSER[1];
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Abstract: PAMs of centrally expressed nicotinic acetylcholine receptors (nAChRs) have therapeutic potential in areas of cognition, motor function and addiction. Nicotine (NIC) addiction is thought to operate through nAChR located primarily on dopaminergic (but also GABAergic) neurons in the mesencephalic ventral tegmental area. Mouse embryonic neurons from the ventral mesencephalon in culture have functional α7 and non-α7 nAChRs (Neurosci 109, 275-285, 2002 ; FASEB J. fj.11-182824, 2011) and contain dopaminergic neurons. In the present study we investigated the effects of PAMs at α7 and α4β2* nAChR on ACh- and NIC-induced changes in [Ca2+]i in mouse embryonic (E18) mesencephalic neurons in primary culture. These cultures contained about 10% tyrosine hydroxylase positive neurons. Atropine (1 µM) was present in the experiments that included ACh. NIC (100 µM) had a small effect on [Ca2+]i (17 % increase vs. basal, one neuron out of 35 tested). A higher concentration of NIC (300 µM) was able to recruit more neurons (57%) and to increase the average [Ca2+]i response to 50% over basal. The effect of ACh (100 µM) was greater than that of NIC (100 µM). The PAM α7 PNU 120596 concentration-dependently amplified the effect of NIC 100 µM, by recruiting more neurons and increasing the amplitude of the [Ca2+]i response. The PNU 120596-induced potentiation of NIC was antagonized by the α7 nAChR antagonist methyllycaconitine (MLA, 10-100 nM). Desfomylflustrabromine (dFBr), a PAM α4β2* (1 µM), increased the number of ACh responding neurons and the amplitude of the [Ca2+]i increase, but had little effect on the response to NIC. The α4β2 nAChR antagonist dihydro-β-erythroidine (DHβE, 1 µM) partially antagonized the effect of the high concentration of NIC (300 µM) but did not antagonize ACh even at 10 µM. MLA (30 nM) partially inhibited the effect of NIC. PNU 120596, dFBr, MLA and DHβE by themselves had little or no effect on [Ca2+]i. These data show that nAChR PAMs α7 and α4β2* are able to modulate the activity of embryonic mesencephalic neurons in culture in response to ACh and NIC.

Disclosures: C. Cosi: None. **F. Marty:** None. **P. Maillos:** None. **P.C. Moser:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.10/C28

Topic: B.02. Ligand Gated Ion Channels

Support: NINDS 1R15NS070760-01

HHMI Undergraduate Science Education grant

Title: Does the β2 C loop of the neuronal nicotinic acetylcholine receptor dictate the type of allosteric modulation?

Authors: *M. M. LEVANDOSKI, C. A. SHORT, C. A. SIBBALD, T. KLJAIC;
Grinnell Col., GRINNELL, IA

Abstract: Numerous compounds allosterically modulate neuronal nicotinic acetylcholine receptors (nAChRs). Studying nicotinic receptors heterologously expressed in *Xenopus* oocytes primarily by electrophysiology, we identified a novel binding site for the anthelmintics morantel and oxantel in mammalian nAChRs. The site is in the β(+)/α(–) interface of α3β2 receptors, in a pocket homologous to the canonical ACh/competitive antagonist site. We have elucidated the residues necessary for constituting this binding site and the determinants of its specificity; in particular, the anthelmintics generally potentiate α3-containing receptors but inhibit α4-containing receptors. We hypothesize that flexibility or concerted movement of the C loop of the β2 subunit is important in allosteric modulation from this site, as well as for receptor activation by agonist. Co-expressing mutant β2 subunits with a cysteine substitution in the C loop and a paired α3(–) cysteine mutant yields receptors susceptible to modification by oxidation and reduction. The effects of these chemical treatments on receptor activation depend on the location of the residue pairs. For example, oxidation of α3T115Cβ2D190C receptors significantly reduced evoked currents compared to control, whereas oxidation of α3T115Cβ2S192C significantly enhanced them. In ongoing studies, we aim to determine the extent to which the putative motion in the β2 C loop is analogous to that of the canonical α subunit C loop for agonist activation of nAChRs, and whether contacts made from the β2 C loop dictate the nature of the modulation.

Disclosures: M.M. Levandoski: None. **C.A. Short:** None. **C.A. Sibbald:** None. **T. Kljaic:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.11/C29

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant R01-GM8360

The ALSAM Foundation

Title: Transmembrane structural models of human neuronal nicotinic acetylcholine receptors in unbound, agonist and antagonist bound complexes optimized by extensive molecular dynamics simulations

Authors: *D. XU[1], P. W. TAYLOR[3], H. J. D. MILLER[4], A. TAO[5], T. T. TALLEY[2];
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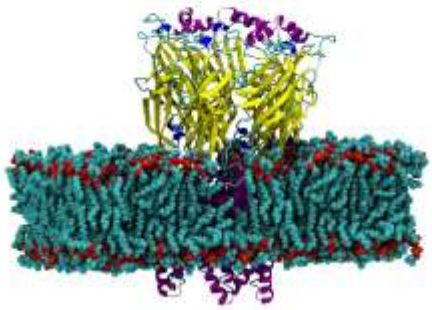
Abstract: Past efforts in modeling human neuronal nicotinic acetylcholine receptors (nAChRs) have been primarily focused on combining structural information from two sources: the X-ray structures of water soluble acetylcholine binding proteins (AChBPs) of snails, and the electron microscopy (EM) derived structures of the transmembrane domain of the Torpedo nAChR. However, this approach has several inherent deficiencies: The structures of Torpedo nAChR and many snail AChBPs, published nearly a decade ago, have less than 30% sequence identities to human nAChRs. Compared to x-ray structures, the quality of EM derived structures suffers from low atomic resolution. On the other hand, AChBP structures do not provide any information about transmembrane domain and might be considered as a distinct subtype of receptor ectodomain.

In recent years, structural biology of the pentameric ligand-gated ion channel family (Cys-loop receptors) has experienced remarkable progress. Based on the recently available X-ray structure determination of C. elegans glutamate-gated chloride channel (GluCl), we constructed three-dimensional models of the extracellular and transmembrane domains embedded within lipid bilayers of three major types of human nAChRs, α7, α4β2, and α3β4, in unbound and complex forms bound to a variety of agonists and antagonists. Large-scale all-atom explicit solvent molecular dynamics (MD) simulations are employed to allow full equilibration and energy minimization. To the best our knowledge, this is a first attempt to present a complete nAChR structure with extracellular and transmembrane domains with annular lipid bilayers. The model employs crystallographic structural resolution and is optimized via extensive MD simulations. We anticipate that such nAChR models will provide an improved structural base for understanding the molecular determinants of conformational states accompanying ligand binding to nAChRs.



Disclosures: D. Xu: None. **P.W. Taylor:** None. **H.J.D. Miller:** None. **A. Tao:** None. **T.T. Talley:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.12/C30

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant DA14241

NIH Grant DA033597

NIH Grant GM18360

ASLAM foundation

NIH Grant P20RR016467

Title: Nicotinic acetylcholine receptor (nAChR) modulators based on the scaffold 3,7-diazabicyclo[3.3.1]nonane

Authors: I. TOMASSOLI[1], C. EIBL[1], J. WU[2], K.-Y. HO[2], E. VALDAMBRINI[2], M. R. PICCIOTTO[3], R. L. PAPKE[4], P. TAYLOR[2], T. T. TALLEY[5], *D. GUNDISCH[1];
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Abstract: 3,7-diazabicyclo[3.3.1]nonane is a naturally occurring scaffold (known as bispidine), which is a moiety found e.g. in the nicotinic acetylcholine receptor (nAChR) ligand cytisine and the sodium ion channel blocker sparteine. Bispidine derivatives have been investigated as novel nAChR compounds, but have also been examined as the base of potential antiarrhythmic drugs, opioid receptor ligands, antiviral agents, for BIOS, and as multidentate ligands for organometallic agents. We used bispidine in development of two nicotinic compound series based on the 1) template, and 2) hybrid approach. The template approach involved the examination of linker/spacer motifs, whereas the hybrid approach was based on the overlap mode of two identical or distinct pharmacological entities. Compounds were evaluated for their affinities for diverse nAChR subtypes by competition with the radioligands [3H]epibatidine (a4/b2*, a3/b4* and muscle type) and [3H]methyllycaconitine (a7*), in membrane fractions from

rat brain, pig adrenal, and Torpedo californica electroplax. In addition, [3H]epibatidine was used in radioligand competition assays for testing selected candidates of the compound series at three different acetylcholine binding proteins (Lymnaea stagnalis, Aplysia californica (Ac) and a single Ac mutant) as structural surrogates for nAChRs. A broad spectrum of affinities (Ki values: < 1 nM to > 10,000 nM) was obtained providing important insights into structure-affinity relationships for these nAChR targets. High resolution X-ray crystal structures of the compounds in complex with both WT and mutant AChBPs allows a detailed examination of the molecular features contributing to specificity and potency. Both drug design approaches are successful strategies for the development of novel nAChR ligands, including low-efficacy partial agonists at a4/b2* nAChRs with reduced off-target (a3/b4*, a7) and 5-HT3 activity.

Disclosures: I. Tomassoli: None. **C. Eibl:** None. **J. Wu:** None. **K. Ho:** None. **M.R. Picciotto:** None. **R.L. Papke:** None. **P. Taylor:** None. **T.T. Talley:** None. **D. Gundisch:** None. **E. Valdambrini:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.13/C31

Topic: B.02. Ligand Gated Ion Channels

Support: RO1-GM18360 (PT)

ALSAM foundation (TT)

Title: Structure-activity relationships of selected natural products and template designed ligands for acetylcholine binding proteins (achbp), nicotinic acetylcholine receptors (nachrs) and related ligand-gated ion channels

Authors: T. T. TALLEY[1], J. WU[2], J. G. YAMAUCHI[2], A. NEMECZ[2], K. GOMEZ[2], A. SERRANO[2], K.-Y. HO[2], *P. TAYLOR[3];
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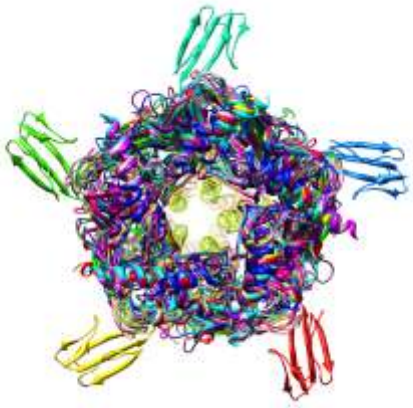
[2]Skaggs Sch. of Pharm. & Pharmaceut. Sciences, Univ. of California, San Diego, La Jolla, CA;
[3]Pharmacol., UC San Diego, LA JOLLA, CA

Abstract: We have employed the AChBP as a template lead into studying the molecular recognition properties of the pentameric ligand-gated ion channels. The approach encompasses direct measures ligand association with AChBP, delineating agonist and antagonist activity at subtypes of nAChR on intact cells, and in limited cases, crystallographic structures complexes of

the ligands with AChBP to ascertain the binding poses of the associated ligand. Responses for human α7 and α4β2 nAChRs as well as mouse 5-HT3 A pLGICs were characterized using an in vitro fluorescent functional assay using a genetically encoded Ca2+ indicator. Compounds were identified and characterized as agonists or antagonists on the basis of peak Förster resonance energy transfer responses mediated through Ca2+ flux through the pLGICs. This three pronged approach has enabled us to use structure to design and define molecular determinants of selectivity. For example, the binding poses and determinants of selectivity distinguish quaternary nicotinic ligands stabilized in an aromatic nest of side chains can be distinguished from imines and secondary and tertiary amines where a hydrogen bond from a protonated nitrogen to a backbone carbonyl oxygen becomes the critical orientation of the bound ligand. In turn, positions of the less basic amines are defined within the subunit interfaces through the association with polar residues and water. In turn the binding protein serves as a template for synthesis and characterization of unique ligands that achieve receptor subtype selectivity.



Disclosures: T.T. Talley: None. **P. Taylor:** None. **J. Wu:** None. **J.G. Yamauchi:** None. **A. Nemecz:** None. **K. Gomez:** None. **A. Serrano:** None. **K. Ho:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.14/C32

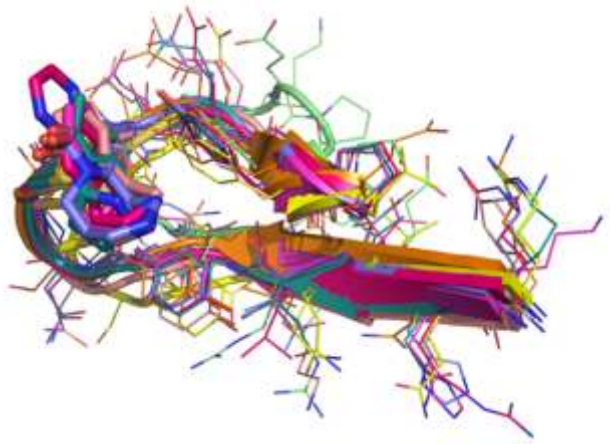
Topic: B.02. Ligand Gated Ion Channels

Support: ALSAM Foundation

Title: Development of heteromeric human nicotinic receptor/AChBP chimeras

Authors: D. HENDRICKSON[1], M. WILSON[1], P. TAYLOR[2], *T. T. TALLEY[1];
[1]Dept. of Biomed. and Pharmaceut. Sci., Idaho State Univ. Col. of Pharm., Meridian, ID;
[2]Skaggs Sch. of Pharm. & Pharmaceut. Sciences, Univ. of California, San Diego, La Jolla, CA

Abstract: The acetylcholine binding proteins (AChBPs) have provided a wealth of information on structure of the extracellular domain of the Cys-loop ligand-gated ion channels since their initial report by Sixma and colleagues. The availability of high resolution X-ray crystal structures of these proteins in complex with various nicotinic ligands has provided an atomic resolution view of the determinants of ligand recognition. In turn, this has provided opportunities for structure-guided drug design, target “template” synthesis and computational analyses of ligand recognition. However, these efforts had been hampered by the fact that the binding proteins, while homologous with human nicotinic receptors, have a pharmacology that is dissimilar to human drug targets. In addressing this shortcoming we have successfully designed a sires chimeric AChBP constructs. The initial set of constructs replaced the amino acids in the C-loop of the ligand binding site with those corresponding to each of the human alpha subunits. With the successful expression, characterization and co-crystallization of the initial chimeric entities we have expanded our efforts to include non-alpha mutations with the goal of developing constructs that better mimic heteromeric binding environments. The new series of human nicotinic receptor/AChBP chimeras allow for detailed examination of the molecular determinants of ligand recognition and selectivity. Using these insights we are developing new ligands targeting specific receptor subtypes.



Disclosures: D. Hendrickson: None. **T.T. Talley:** None. **M. Wilson:** None. **P. Taylor:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.15/C33

Topic: B.02. Ligand Gated Ion Channels

Support: James and Esther King Biomedical Research grant KG12

NIH Grant GM57481

P20RR016467

Title: Differential modulation of brain nAChR function by cytisine and bispidine compounds and emergent properties of a hybrid molecule

Authors: *C. PENG[1], I. TOMASSOLI[2], C. EIBL[2], D. GUENDISCH[2], R. L. PAPKE[1]; [1]Dept. of Pharmacol. and Therapeut., Col. of Medicine, Univ. of Florida, Gainesville, FL; [2]Dept. of Pharmaceut. Sci., Col. of Pharmacy, Univ. of Hawaii, Hilo, HI

Abstract: Partial agonist therapies for the treatment of nicotine addiction and dependence depend on both agonistic and antagonistic effects of the ligands, and side effects associated with other nAChRs greatly limit the efficacy of nicotinic partial agonists. Here, we evaluated the *in vitro* pharmacological properties of four partial agonists, two current smoking cessation drugs varenicline and cytisine, as well as two novel bispidine compounds BPC and BMSP, by using defined nAChR subtypes expressed in *Xenopus* oocytes. All four agents showed relatively little activity for mouse muscle α1β1εδ nAChR, although 30 μM BPC produced a small inhibition when co-applied with control ACh. Like varenicline and cytisine, BPC was a partial agonist of α4β2 nAChRs, especially of the HS form. However, BMSP produced very little activation of these α4β2 receptors. The partial agonist activity of BPC for α4β2α5 was comparable to cytisine, while BMSP was the least efficacious among the four agents on this receptor as well as β3α4β2α6β2. Unlike varenicline and cytisine, BPC and BMSP showed desired low activity for the two off-target subtypes, α7 and α3β4. In acute co-application experiments, HS α4β2 receptors were the most sensitive to all four agents, although least sensitive to BPC. All four agents inhibited α4β2α5 and β3α4β2α6β2 receptors which were most sensitive to varenicline and cytisine. Several α4* subtypes were sensitive to all four agents when pre-applied for 5 minutes prior to ACh application, with the greatest effects obtained with the HS α4β2. In addition, bath applications of BMSP at submicromolar concentrations effectively down-regulated the responses of all α4* receptors other than the α6* subtype. All α4* receptors were also functionally down-regulated by bath-applied 100 nM BPC, which produced mecamylamine-sensitive steady-state activation that was not evident with BMSP. Modulation of α4*- and α7-mediated responses in rat lateral geniculate nucleus (LGN) neurons and hippocampal stratum radiatum (SR) interneurons by the four partial agonists were also investigated. The LGN neurons were sensitive to a very low concentration of varenicline, and the SR interneuron responses were also sensitive to varenicline at a submicromolar concentration. Both types of neurons were approximately 10-fold less sensitive to bath applications of cytisine. While 300 nM BPC strongly inhibited the ACh-

evoked responses of LGN neurons, it didn't suppress the α7 currents of SR interneurons. Similar results were observed with 300 nM BMSP. Our data indicate that BPC and BMSP are promising α4β2* partial agonists for pharmacotherapeutics.

Disclosures: C. Peng: None. **I. Tomassoli:** None. **C. Eibl:** None. **D. Guendisch:** None. **R.L. Papke:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.16/C34

Topic: B.02. Ligand Gated Ion Channels

Title: Positive allosteric modulation of $(\alpha4)3(\beta2)_2$ nicotinic acetylcholine receptors by NS9283 *In vitro* and *In vivo*

Authors: *M. GRUNNET[1], J. BASTLUND[1], M. SARTER[2], A. JENSEN[3], M. GRUPE[1]; [1]Lundbeck A/S, Valby, Denmark; [2]3Department of Psychology and Neurosci. Program, Univ. of Michigan, Ann Arbor, MI, USA, Ann Arbor, MI; [3]Dept. of drug design and pharmacology, Copenhagen, Denmark

Abstract: Disturbance of cholinergic transmission is implicated in a series of neurological conditions such as ADHD, schizophrenia, Parkinson's Disease and Alzheimer's Disease. The nicotinic acetylcholine receptors (nAChRs) are important players in cholinergic transmission. nAChRs are cation-selective ligand-gated ion channels belonging to the superfamily of Cys-loop receptors. Among many different subtypes α4β2 nAChR is believed to be a key player in neuronal synaptic communication. Within the field of drug development targeting nAChRs it has been proposed that positive allosteric modulators (PAMs) possess several pharmacological advantages over agonists. These include higher subtype selectivity as well as maintenance of endogenous spatiotemporal patterns of cholinergic signaling due to lack of desensitizing effects as observed with agonists. The compound NS9283, represent a potent and selective PAM of α4β2 nAChRs. Previous patch-clamp studies have demonstrated how NS9283 increase agonist-induced activity of α4β2 nAChRs by left-shifting the concentration-response curve ~60-fold through a decrease in the rate of deactivation but without major effect on desensitization parameters. Furthermore, systemic administration of NS9283 potentiates nicotine-evoked glutamate release in rat medial prefontal cortex *in vivo*. In the present study we have investigated the impact of nicotinic allosteric modulation on auditory evoked potentials in rats performing a two-tone auditory discrimination task using NS9283. This was addressed with EEG electrodes

implanted into secondary auditory cortex, mediodorsal thalamus, hippocampus and prefrontal cortex. Especially the latter two are key brain regions for higher cognitive executive functions, such as working memory and attention.

Disclosures: M. Grunnet: None. **J. Bastlund:** None. **M. Sarter:** None. **A. Jensen:** None. **M. Grupe:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.17/D1

Topic: B.02. Ligand Gated Ion Channels

Support: CTI Switzerland

Title: A walk in the chemical space to discover new molecules active at nAChRs

Authors: J. BÜRGI[1], *S. BERTRAND[2], E. NEVEU[3], T. SCHAER[3], J.-L. REYMOND[1], D. BERTRAND[3];
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Abstract: The chemical space can be seen as an almost infinite universe in which each point represents one possible combination of atoms forming a defined molecule. Based on the observation that compounds comprising a quinuclidine moiety, such as the PNU-282987 are potent agonists at the neuronal nicotinic acetylcholine receptors (nAChRs) a strategy of virtual screening was developed to identify interesting analogues. Herein we report N-(2-halobenzyl)-(S)-3-aminoquinuclidine (S)-6a-c as a new class of α3β2 nAChR PAM, identified by surveying nearest neighbours of 1 within the chemical space of the public database ChEMBL using a web-browser available at www.gdb.unibe.ch. We term this approach "chemical space walking" because the vicinity of most compounds comprises at most a few hundred nearest neighbours when focusing on known molecules, allowing one to perform the selection without the aid of a machine. Identified compounds were screened for functional effects with automated two electrode voltage clamp on approach on human recombinant nicotinic acetylcholine receptors expressed in Xenopus oocytes using HiClamp (Multichannel system). Pharmacological characterization further highlighted the properties of the molecules of interest and confirmed that ligands (S)-6a-c are the strongest α3β2 nAChR PAM to date. This approach confirms that chemical space walking should be generally useful to identify bioactive drug analogues.

Disclosures: J. Bürgi: None. **S. Bertrand:** A. Employment/Salary (full or part-time):; hiqscreen. **E. Neveu:** A. Employment/Salary (full or part-time):; hiqscreen. **T. Schaer:** A. Employment/Salary (full or part-time):; hiqscreen. **J. Reymond:** None. **D. Bertrand:** A. Employment/Salary (full or part-time):; hiqscreen.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.18/D2

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant DA019375

NIH Grant DA015663

Title: Desensitization of presynaptic alpha4beta2- and alpha4alpha5beta2-nAChR

Authors: *S. R. GRADY, C. R. WAGEMAN, M. J. MARKS;
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Abstract: Previously we have shown that presynaptic nicotinic acetylcholine receptors (AChR) of different subtypes on dopaminergic terminals differ in desensitization. The alpha4alpha5beta2-nAChR subtype requires higher concentrations of nicotine than the alpha4beta2-subtype for an equivalent amount of desensitization with a 10 min exposure. Experiments were conducted to compare the 10 min exposure with 60 min exposures. No additional increase in desensitization was seen with the longer exposure for either subtype. Recovery kinetics and amount of recovery were also unchanged.
To model longer term exposure more equivalent to smoking exposures, we treated mice with drinking water containing nicotine (dose) for several weeks. We compared these mice to mice given tap water. One striatum from each mouse was prepared in the normal fashion and the other side in buffers containing 300 nM nicotine for all steps of crude synaptosome preparation and [3H]-dopamine uptake. This protocol results in about 90 min of nicotine exposure. Aliquots were superfused with buffer with or without 300 nM nicotine, followed by release stimulation using acetylcholine. The procedure results in 8 groups of samples: two treatment groups X two preparation groups X two superfusion groups. Results indicated that the longterm treatment did not significantly affect subsequent desensitization in this assay.
Because the alpha4alpha5beta2-nAChR subtype appears to be significantly more active than the alpha4beta2-nAChR subtype, there is some possibility that this higher activity could create a spare receptor population. We investigated this possibility by using subunit null mutant

heterozygous mice that have been shown to express about half as much receptor as wildtype mice. The beta2 heterozygous mice exhibited about a 40% decrease in activity measured by [3H]-dopamine release, while the alpha4 heterozygous mice had approximately 20% decrease. Results with the beta2 or the alpha4 heterozygous mice with the 10 min desensitization protocol were not different from wildtype, suggesting a lack of spare receptors.

Disclosures: S.R. Grady: None. **C.R. Wageman:** None. **M.J. Marks:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.19/D3

Topic: B.02. Ligand Gated Ion Channels

Support: CNRS-DRI PICS grant (to YB and PM)

USPHS grants (to PT)

ANR grants (to JM)

NIH/NIGMS grants (to AZ)

Title: Structural determinants in AChBP and nicotinic ligands conferring high affinity binding, nAChR subtype selectivity, and antagonist or full or partial agonist profiles

Authors: *Y. BOURNE[1], S. B. HANSEN[2], R. E. HIBBS[2], G. SULZENBACHER[1], Z. RADIC[2], R. ARAOZ[3], T. T. TALLEY[2], T. HUXFORD[2], E. BENOIT[3], J. SHI[2], M. REYNAUD[4], W. R. KEM[5], A. ZAKARIAN[6], D. SERVENT[4], J. MOLGO[3], P. TAYLOR[2], P. MARCHOT[1]; [1]AFMB (CNRS/AMU), Marseille Cedex 09, France; [2]Pharmacol., UCSD/SSPPS, La Jolla, CA; [3]INAF (CNRS), Gif-sur-Yvette, France; [4]SIMOPRO, iBiTecS, CEA, Gif-sur-Yvette, France; [5]Pharmacol. and Therapeut., UF, Gainesville, FL; [6]Chem. and Biochem., UCSB, Santa Barbara, CA

Abstract: The pentameric acetylcholine-binding protein (AChBP) from marine and freshwater snails is a soluble surrogate of the extracellular, ligand-binding domain of the nicotinic acetylcholine receptor. Nicotinic agonists and competitive antagonists bind primarily within a nest of aromatic side chains contributed by loops C and F on opposing faces of each subunit interface. We have solved a range of crystal structures of Lymnaea and Aplysia AChBP in complexes with peptidic and organic antagonists, including the emergent marine phycotoxins, and with organic full and partial agonists of various plant, animal and synthetic origins. We show

that common AChBP determinants are involved into conferring high affinity binding whereas distinctive determinants, located within the nest or extending outside the nest towards apical, radial or “membrane” subsites of the interface, and associated with positional/conformational changes in loop C, appear to be associated with agonist/antagonist-elicited modulation of channel opening in the full-length receptor. These studies also point to the respective contributions of loops C and F in AChBP and determinants in the ligand in dictating either broad or narrow selectivity for muscle-type or neuronal receptor subtypes. A comparative overview of binding (kinetic and equilibrium approaches), functional (voltage-clamp recordings), and structural (x-ray crystallography) data will be presented.

Bourne Y, Talley TT, Hansen SB, Taylor P, Marchot P (2005) Crystal structure of a Cbtx-AChBP complex reveals essential interactions between snake alpha-neurotoxins and nicotinic receptors. EMBO J 24, 1512-22.

Hansen SB, Sulzenbacher G, Huxford T, Marchot P, Taylor P, Bourne Y (2005) Structures of Aplysia AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations. EMBO J 24, 3635-46.

Hibbs RE, Sulzenbacher G, Shi J, Talley TT, Conrod S, Kem WR, Taylor P, Marchot P, Bourne Y (2009) Structural determinants for interaction of partial agonists with acetylcholine binding protein and neuronal alpha7 nicotinic acetylcholine receptor. EMBO J 28, 3040-51.

Bourne Y, Radic Z, Aráoz R, Talley TT, Benoit E, Servent D, Taylor P, Molgo J, Marchot P (2010) Structural determinants in phycotoxins and AChBP conferring high affinity binding and nicotinic AChR antagonism. Proc Natl Acad Sci USA 107, 6076-81.

Bourne Y, Sulzenbacher G, Radic Z, Aráoz R, Reynaud M, Benoit E, Servent D, Zakarian A, Taylor P, Molgó J, Marchot P, in preparation.

Disclosures: Y. Bourne: None. **S.B. Hansen:** None. **R.E. Hibbs:** None. **G. Sulzenbacher:** None. **Z. Radic:** None. **R. Araoz:** None. **T.T. Talley:** None. **T. Huxford:** None. **E. Benoit:** None. **J. Shi:** None. **M. Reynaud:** None. **W.R. Kem:** None. **A. Zakarian:** None. **D. Servent:** None. **J. Molgo:** None. **P. Taylor:** None. **P. Marchot:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

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Program#/Poster#: 224.20/D4

Topic: B.02. Ligand Gated Ion Channels

Support: NIH grant DA027990

NIH grant DA32489

NIH grant DA25947

Title: Pharmacological properties of novel 2,5-disubsituted-pyridinyl compounds as highly selective nicotinic acetylcholine receptor desensitizers

Authors: *Y. XIAO[1], Y. LIU[2], E. TUAN[1], N. SAHIBZADA[1], N. AL-MUHTASIB[1], T. TRAN[1], T. XIE[1], M. PAIGE[2], M. L. BROWN[2], A. H. REZVANI[3], E. D. LEVIN[3], K. J. KELLAR[1]; [1]Dept Pharmacol and Physiol, [2]Ctr. for Drug Discovery, Georgetown Univ., WASHINGTON, DC; [3]Psychiatry and Behavioral Sci., Duke Univ. Med. Ctr., Durham, NC

Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) are essential for a wide range of physiological and behavioral functions. They are implicated in a number of pathological processes and are the targets of nicotinic drugs. It is widely accepted that there are three functional states of nAChRs: closed, open and desensitized. We reported previously that Sazetidine-A potently and selectively desensitizes α4β2 nAChRs. It was proposed that selective ligands causing long lasting nAChR desensitization might be used as therapeutics for treating CNS disorders. Since then, studies have demonstrated significant behavioral effects of Sazetidine-A in several animal models including reducing nicotine and alcohol self-administration. Recently, we reported on the chemistry and pharmacology of 2,5-disubsituted-pyridinyl analogs of Sazetidine-A (Yong Liu et al., 2013, JMC), which selectively desensitize α4β2 nAChRs in cell models and significantly reduce alcohol intake in alcohol preferring rats. Importantly, preliminary results from studies in a ferret model indicate that the novel analogs have an improved adverse side-effect profile in comparison with that of varenicline. More recently, we have completed in vitro pharmacological studies of a new compound, YL-2-203, which is a member of this novel class of selective α4β2 nAChR ligands. YL-2-203 binds to human α4β2 nAChRs with much higher affinity (Ki = 0.2 nM) than to human α3β4 nAChRs (Ki = 97 nM). It shows very low agonist activity at both receptor subtypes. It potently and selectively desensitizes human α4β2 nAChRs (IC50(10') = 44 nM). This new nicotinic compound is currently being studied in a rat model of nicotine self-administration as well as in other animal behavioral models.

Disclosures: Y. Xiao: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor of patents. **Y. Liu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor of patents. **E. Tuan:** None. **N. Sahibzada:** None. **N. Al-Muhtasib:** None. **T. Tran:** None. **T. Xie:** None. **M. Paige:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor of patents. **M.L. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor of patents. **A.H. Rezvani:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor of patents. **E.D. Levin:** E. Ownership Interest (stock, stock

options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor of patents. **K.J. Kellar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventor of patents.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.21/D5

Topic: B.02. Ligand Gated Ion Channels

Support: Alzheimer's Resource of Alaska

Title: Destabilization of a desensitized state contributes to potentiation of low-sensitivity α4β2 nicotinic receptors by desformylflustrabromine

Authors: A. DEMMERLY, *B. W. EDMONDS;
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Abstract: Neuronal nicotinic acetylcholine receptors (nnAChRs) are promising drug targets for therapy of Alzheimer's disease, nicotine addiction, and other disorders of cholinergic transmission. In comparison to partial agonists, noncompetitive (allosteric) modulators of nicotinic receptors may preserve the normal pattern of receptor activation by endogenous ligand (ACh), and therefore be well suited to correct errors in cholinergic signaling. Desformulflustrabromine (dFBr) is a selective, noncompetitive modulator of the α4β2 class of nAChRs. α4 and β2 subunits assemble to form receptors with two, alternate stoichiometries that exhibit high- and low-sensitivity to agonist, respectively. The heterogeneity of α4β2 nAChRs creates challenges for investigations of receptor mechanisms using whole-cell recording methods. We now use the cell-attached patch-clamp method to examine the effects of dFBr on gating of isolated low-sensitivity (30 pS) receptors expressed in HEK-293 cells. Open and closed duration distributions obtained with 1 μM ACh were fitted (QuB Software) using a 7-state model that includes two binding steps, mono- and di-liganded open states (O1 and O2, respectively), and two long-lived closed (desensitized) states, D1 and D2, that communicate with the di-liganded closed and open states, respectively. In 1 μM dFBr (and 1 μM ACh) we observe two noteworthy effects. First, the mean lifetime of D1 is decreased by a factor of 53 (mean lifetime in control = 390 ms; mean lifetime in dFBr = 7.4 ms). Second, the relative frequency of short (mean lifetime ≈ 1 ms), mono-liganded to long (mean lifetime ≈ 10 ms), di-liganded openings shifts from 30% in control to 80% in dFBr. This shift arises primarily from an apparent 45-fold

increase in the rate of entry into the mono-liganded open state. Simulations of macroscopic currents yield potentiated currents at all ligand concentrations, and the expected EC_{50} values were 49 μM for control, and 10.5 μM for dFBr. For very low concentrations of ACh (~ 100 nM), simulations are consistent with the view that potentiation is primarily due to the increased rate of entry into O1. For agonist concentrations above 100 nM, potentiation arises almost exclusively from destabilization of D1.

Disclosures: A. Demmerly: None. **B.W. Edmonds:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.22/D6

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant DA012976

Title: Assessing alternate stoichiometries of the α4β2 neuronal nicotinic acetylcholine receptor

Authors: *K. DEDOMINICIS, N. SAHIBZADA, T. TRAN, Y. XIAO, B. WOLFE, K. KELLAR, R. YASUDA;
Pharmacol., Dept. of Pharmacology/Georgetown Univ., Washington, DC

Abstract: The nicotinic acetylcholine receptor (nAChR) plays a key role in nicotine addiction. One of the most important nAChRs related to nicotine addiction and withdrawal symptoms is the α4β2 nAChR. In heterologous expression systems, α4β2 nAChRs exist in one of two stoichiometries: (α4)3(β2)2 or (α4)2(β2)3. These different stoichiometric forms have been termed low-sensitivity (LS) or high-sensitivity (HS) α4β2 nAChRs, respectively, due to observed differences in potency in response to acetylcholine (ACh). Based on the literature, full concentration-response curves to ACh can indicate whether α4β2 nAChRs expressed heterologously are HS, LS, or a mixture of both stoichiometries. In this work, we altered transfection ratios of α4:β2 cDNA from 1:6 to 4:1 to favor assembly of HS or LS nAChRs. We present two new methods to predict which stoichiometric form(s) of rat α4β2 nAChRs is/are expressed in these cells. The first method uses whole-cell voltage clamp electrophysiology to predict the predominance of HS or LS α4β2 nAChRs based on peak current amplitude responses to discrete concentrations of ACh. We demonstrate that the HS stoichiometry has similar responses to 10 and 1000 μM ACh. In contrast, in the LS stoichiometry the response to 10 μM ACh is only 10% that of 1000 μM ACh. This is consistent with the complete concentration-response curves of the two stoichiometric forms. The second method uses immunoblotting

techniques to directly quantify and compare the ratio of α4 to β2 nAChR subunits in harvested tissue using an α4-β2 dimer construct as a standard. We use immunoprecipitation and/or chromatographic methods to remove subunit assemblies that are smaller than pentamers. By developing these assays to determine the stoichiometry of α4β2 nAChRs in HEK cells, we plan to assess the native stoichiometry of the α4β2 nAChR in brain from both naïve and nicotine treated animals.

Disclosures: K. Dedominicis: None. **N. Sahibzada:** None. **T. Tran:** None. **Y. Xiao:** None. **B. Wolfe:** None. **K. Kellar:** None. **R. Yasuda:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.23/D7

Topic: B.02. Ligand Gated Ion Channels

Support: DFG SFB 581 TP B27

DFG HE 2621/4-2

Title: Contribution of the two binding sites in gating nicotinic receptor channels

Authors: *M. HECKMANN[1], P. STOCK[1], D. LJASCHENKO[1], J. DUDEL[2];
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Abstract: Muscle type nicotinic acetylcholine receptor/channels (nAChRs) bind ligands at interfaces of α- and neighbouring γ- or δ-subunits. αγ- and αδ-sites differ in affinity but their contributions in opening the channel remain elusive. We compared high resolution patch-clamp currents evoked by epibatidine (Ebd), carbamylcholine (CCh) and acetylcholine (ACh). Ebd binds with higher affinity at αγ- than at αδ-sites whereas CCh and ACh prefer αδ-sites. Short (τ_{O1}), intermediate (τ_{O2}) and long types of openings (τ_{O3}) were observed with all three agonists. Below 10 nM Ebd preferentially τ_{O2} openings occurred from binding at αγ-sites. Vice versa, τ_{O1} openings appear to be generated at αδ-sites. In addition two types of bursts appeared: Short bursts of on average 0.75 ms (τ_{B1}) that should also arise from the αγ-site, and long bursts of 12 to 25 ms (τ_{B2}) durations arising from double liganded receptors. The duration of the about 180 µs openings within these bursts depended on the agonist, but with further improved time resolution may approach the shorter τ_{O2}. The 3 µs closings within bursts appeared to be agonist independent, and like the types of openings to be inherent properties of the channel opening

mechanism. Blocking αδ-sites with α-conotoxin M1 (CTx) eliminated both τ_{O1} and τ_{B2} and left only the longer single τ_{O2} openings and the short τ_{B1} bursts, as expected. When CTx was applied to `embryonic´ mouse endplates, monoquantal current amplitude and decay time constants were reduced and rise times increased, as expected.

Disclosures: M. Heckmann: None. **P. Stock:** None. **D. Ljaschenko:** None. **J. Dudel:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

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Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant DA027990

NIH Grant DA32489

NIH Grant DA25947

Title: Functional properties of classic and novel nicotinic ligands: A patch clamp study using DynaFlow rapid solution exchange system

Authors: *E. TUAN[1], Y. LIU[2], T. XIE[1], N. AL-MUHTASIB[1], T. TRAN[1], M. PAIGE[1], M. BROWN[2], N. SAHIBZADA[1], K. J. KELLAR[1], Y. XIAO[1];
[1]Pharmacol., [2]Drug Discovery Program, Georgetown Univ., Washington, DC

Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) are cys-looped, pentameric ligand-gated ion channels that open in response to acetylcholine. These receptors are assembled in both homomeric and heteromeric fashions from a range of subunits, including nine α subunits and three β subunits. To study pharmacological properties of human nAChRs, we have established two stable HEK293 cell lines: YXα4β2H1, which expresses the human α4β2 subtype, and YXα3β4H1, which expresses the human α3β4 subtype. In $^{86}Rb^{+}$ efflux assays, acetylcholine potently activates nAChRs expressed in the cells. Compared to acetylcholine, nicotine elicits 85% agonist efficacy at the human α4β2 nAChRs and 100% efficacy at the human α3β4 nAChRs. To further explore the function of these two nAChR subtypes, we used the Dynaflow rapid solution exchange system to administer drugs to single cells while measuring elicited whole cell currents with patch clamp electrophysiology. Consistent with results from $^{86}Rb^{+}$ efflux assays, nicotine showed 100% agonist efficacy at the human α4β2 nAChRs and 97% efficacy at the human α3β4 nAChRs when determined using patch clamp. In the whole cell

current measurements, Sazetidine-A shows only 3.5% agonist efficacy at rat α3β4 nAChRs, consistent with our previous reports that Sazetidine-A has little efficacy in activating rat α3β4 nAChRs. Interestingly, in contrast to the effects at rat α3β4 nAChRs, Sazetidine-A showed 82% agonist efficacy at human α3β4 nAChRs, indicating a significant interspecies difference between rat α3β4 nAChRs and their human counterparts. We then studied agonist activities of two novel selective α4β2 nAChR ligands, YL-1-127 (Yong Liu et al., 2013, JMC) and YL-2-203 (Xiao et al., this meeting) using the Dynaflow system. The two new ligands have very low efficacy at human α3β4 nAChRs: 1.3% by YL-1-127 and 5.7% by YL-2-203. These novel ligands should be further studied for developing new CNS therapeutics.

Disclosures: E. Tuan: None. **Y. Liu:** None. **T. Xie:** None. **N. Al-Muhtasib:** None. **T. Tran:** None. **M. Paige:** None. **M. Brown:** None. **N. Sahibzada:** None. **K.J. Kellar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent. **Y. Xiao:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.25/D9

Topic: B.02. Ligand Gated Ion Channels

Support: NIH GM103801

NIH GM48677

Title: Positional-scanning analysis of α-conotoxin residues that confer selectivity for α3β2 nicotinic acetylcholine receptors

Authors: *A. J. HONE[1,3], S. EBERHARD[2], M. RUIZ[2], M. SCADDEN[2], J. GAJEWIAK[2], A. ALBILLOS[3], J. MCINTOSH[2,4];
[1]Neurosci., [2]Biol., Univ. Utah, SALT LAKE CTY, UT; [3]Pharmacol. and Exptl. Therapeut., Univ. Autonoma de Madrid, Madrid, Spain; [4]Psychiatry, George E. Whalen Veterans Affairs Med. Ctr., Salt Lake City, UT

Abstract: Nicotinic acetylcholine receptors (nAChR) containing α3 subunits mediate fast synaptic transmission between neurons of the parasympathetic, sympathetic, and enteric nervous systems; the receptors subtypes expressed are primarily of the α3β2 and α3β4 subtypes. In the

central nervous system, α3β2 receptors may be expressed in brain regions where the closely related α6β2 subtype is also expressed. Some of these areas include the superior colliculus, interpeduncular nucleus, and the striatum. Distinguishing α3β2 from α6β2 receptors using pharmacological probes has been hampered by the scarcity of ligands that selectively target α3β2 vs α6β2 receptors. Several α-conotoxins (α-Ctx) and their analogs selectively inhibit α6β2 receptors over α3β2 receptors but only a few show a preference for α3β2 over α6β2 receptors and include, for example, OmIA and LtIA. However, these α-Ctxs are only marginally selective for the α3β2 subtype and thus have limited utility for distinguishing α3β2 from α6β2 receptors. We used positional-scanning mutagenesis of α-Ctx PeIA, a peptide that targets both α3β2 and α6β2 with equal potencies, and substituted non conserved residues from α3β2-selective α-Ctxs into the sequence of PeIA in order to identify residues that are important for conferring selectivity for α3β2 receptors over α6β2 receptors. Successive substitution of several residues generated analogs of PeIA with improved selectivity. The results of this study should be useful for guiding the synthesis of ligands that selectively target α3β2 nAChRs.

Disclosures: A.J. Hone: None. **S. Eberhard:** None. **M. Ruiz:** None. **M. Scadden:** None. **J. Gajewiak:** None. **A. Albillos:** None. **J. McIntosh:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.26/D10

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant DA012976

Title: AT-1001: Pharmacological profile at rat and human α3β4 and α4β2 nicotinic receptors

Authors: *C. BOWMAN DALLEY[1], A. LEWIN[1], E. TUAN[1], N. AL-MUHTASIB[1], T. TRAN[1], Y. GAO[2], A. G. HORTI[2], K. J. KELLAR[1];
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Abstract: The predominant nicotinic acetylcholine receptor (nAChR) in major sympathetic ganglia and the pineal gland is the α3β4*subtype. In addition, this nAChR subtype is found in important areas of the CNS, where it has been associated with nicotine addiction and withdrawal. A major impediment to studying α3β4*nAChRs has been the lack of a highly selective drug/ligand for use in pharmacological and radioligand binding studies; however, in 2012, AT-1001 was introduced as a highly selective, high affinity α3β4 nAChR antagonist (Toll, Zaveri et

al., 2012). Because of its high selectivity, AT-1001 might be a powerful tool to study the pharmacology and physiology α3β4* nAChRs. Moreover, AT-1001 potently blocked nicotine self-administration in rats (Toll, Zaveri et al. 2012), suggesting that the drug may have clinical applications in the treatment of addiction. There are certain differences between the rat and human β4 subunit that can result in some pharmacological differences (Young, Broad et al., 2007); therefore, we further characterized AT-1001 by measuring the pharmacologic properties of this drug at both the rat and human receptor subtypes expressed in stably transfected HEK cells. Consistent with the previous study (Toll, Zaveri et al. 2012), in our radioligand binding studies AT-1001 displayed ~30-fold selectivity for rat α3β4 over rat α4β2 nAChRs. However, in studies with human receptors AT-1001 displayed ~1800-fold selectivity. This higher selectivity was due primarily to a much higher affinity for human compared to rat α3β4 nAChRs (K_i 0.12 nM vs 7.6 nM), with virtually no difference in the affinities at the α4β2 nAChRs (K_i 217 nM vs 232 nM). Consistent with the previous report (Toll, Zaveri et al., 2012), saturation binding curves with [^{3}H]-epibatidine in the presence and absence of AT-1001 indicated a mixed type of binding inhibition, with both a rightward shift and an apparent decrease in binding maximum of the curves in the presence of AT-1001 in both rat and human receptors. In $^{86}Rb^+$ efflux assays, AT-1001 was ~8-times more potent in inhibiting the response to co-applied nicotine at human compared with rat α3β4 nAChRs (IC_{50} 0.36 μM vs 2.7 μM). Surprisingly, AT-1001 showed 33% - 50% partial agonist activity at α3β4 nAChRs in both $^{86}Rb^+$ efflux assays and patch clamp measurements. Furthermore, in patch clamp measurements with rapid application, AT-1001 showed partial agonist activity (~12%) at α4β2 nAChRs. Preliminary studies indicate that AT-1001 can also desensitize nAChRs.

Disclosures: C. Bowman Dalley: None. **A. Lewin:** None. **E. Tuan:** None. **N. Al-Muhtasib:** None. **T. Tran:** None. **Y. Gao:** None. **A.G. Horti:** None. **K.J. Kellar:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.27/D11

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant DA026627 (to PW)

Barrow Neurological Foundation Funding (to PW)

NIH Grant NS11323 (to JML)

Title: Differential agonist desensitization of HS vs. LS nicotinic receptors

Authors: *P. WHITEAKER[1], J. B. EATON[1], L. M. LUCERO[1], H. STRATTON[1], Y. CHANG[1], J. F. COOPER[2], J. M. LINDSTROM[2], R. J. LUKAS[1];
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Abstract: α4β2 nAChRs are the predominant subtype in the central nervous system, and exist in two pentameric isoforms, with $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$ stoichiometries. These have high and low sensitivity (HS/LS) agonist activation affinities, respectively. Both isoforms share two orthosteric agonist-binding α4/β2 interfaces, but the $(\alpha4)_3(\beta2)_2$ isoform contains an additional agonist binding site at a unique α4/α4 interface. At low agonist concentrations, $(\alpha4)_3(\beta2)_2$ nAChRs produce a small amount of "HS" function mediated by the α4/β2 interfaces. At higher agonist concentrations the distinctive, and much larger, LS response is seen. For most agonists, desensitization concentration dependence is indistinguishable between HS and LS α4β2 nAChRs. However, using both $^{86}Rb^+$ efflux and two-electrode voltage-clamp (TEVC) electrophysiology, we show that it is possible to preferentially pre-desensitize the HS isoform by extended exposure to a low concentration (3 nM) of the highly HS- vs. LS-preferring agonists AMOP-H-OH and A85380. This also abolishes the small "HS" response from $(\alpha4)_3(\beta2)_2$ nAChRs, while leaving the typical LS response relatively intact. To demonstrate the role of the α4/α4 site in this phenomenon, we mutated aromatic amino-acids in the A-D conserved agonist binding loops to alanine. A concatameric construct was used to enforce LS stoichiometry, and to ensure only the LS interface was mutated. Constructs were expressed in *Xenopus* oocytes, and function assessed using TEVC. In all cases, acute stimulation of the mutant receptors showed an increase in the HS:LS function ratio, as expected if LS function is uniquely dependent on the α4/α4 site. Most mutations did not change EC_{50} values of the LS phase, although those in the C-loop resulted in even lower agonist sensitivity. Maximum function of the A- and C-loop LS concatemer mutants was significantly lower, while that of B- and D-loop mutants was similar to the un-mutated construct. Surface expression of the w.t. and mutant constructs was assessed using [^{125}I]mAb295 binding to intact oocytes, and showed no significant differences. Increased absolute HS-phase function per receptor was seen in two cases (α4/α4 W88A, Loop D; W182A, Loop B); this suggests functional interdependence between the α4/β2 and α4/α4 agonist sites. Occupation of the α4/β2 sites following 5 min exposure to 3 nM AMOP-H-OH also causes changes α4/α4 site-mediated function; LS-phase EC_{50} values were consistently right-shifted. These findings will guide further pharmacological and drug-discovery studies.

Disclosures: P. Whiteaker: None. **J.B. Eaton:** None. **L.M. Lucero:** None. **H. Stratton:** None. **Y. Chang:** None. **J.F. Cooper:** None. **J.M. Lindstrom:** None. **R.J. Lukas:** None.

Poster

224. **"Nicotinic Receptors: Structure, Function, and Modulation I"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 224.28/D12

Topic: B.02. Ligand Gated Ion Channels

Support: NIH PD30DA015663

NIH 5U19DA019375

Title: Novel nicotinic agonist TI-299423 potently elicits conditioned-place preference in wild-type mice

Authors: *T. WALL[1], B. N. COHEN[1], C. R. WAGEMAN[2], S. R. GRADY[2], M. J. MARKS[2], B. H. HENDERSON[1], S. MCKINNEY[1], P. DESHPANDE[1], D. YOHANNES[3], H. A. LESTER[1]; [1]Caltech, Pasadena, CA; [2]Inst. Behav. Genet., UC, Boulder, CO; [3]Targacept, Inc., Winston-Salem, NC

Abstract: Because they are selectively expressed in dopaminergic neurons, nicotinic acetylcholine receptors (nAChRs) containing α6 subunits (α6*) play a key role in nicotine reward and nicotine neuroprotection in Parkinson's disease. Activation of the locomotor response in transgenic mice expressing hypersensitive α6* (α6L9'S*) nAChRs provides a way to test whether nicotinic ligands activate α6* nAChRs. We measured the locomotor response to three novel ligands (TI-299423,TI-165179,TI-186079) to determine whether they activate α6L9'S* nAChRs as potently as nicotine or varenicline. At 500 nmol/Kg, none of the drugs induced locomotor activity in WT mice. In α6L9'S* mice, however, nicotine, varenicline, and TI-299423 (but not TI-165179 or TI-186079) significantly ($P < 0.05$) increased locomotion. Varenicline and TI-299429 had nearly equal effects that were significantly greater than nicotine. Thus, TI-299423 and varenicline potently activated α6L9'S* nAChRs. The nicotinic antagonist mecamylamine (1 mg/Kg) and the dopamine D1 antagonist SCH-23390 blocked (2 mg/Kg) the α6L9'S* locomotor response to TI-299423 (500 mg/Kg). Transgenic mice expressing hypersensitive α4* (α4L9'A*) nAChRs (but not α6L9'S* mice) are hypersensitive to nicotine-induced hypothermia, suggesting that non-α6* nAChRs mediate this effect. To determine whether TI-299423 could selectively activate α6* nAChRs, we compared its potency in inducing hypothermia in α4L9'A*mice with nicotine. At 100 nmol/Kg, nicotine induced a 2 °C drop in core body temperature. TI-299423 did not significantly affect core temperature. At 500 nmol/Kg, TI-299423 significantly reduced core temperature. Thus, TI-299423 appeared to show some selectivity for α6* nAChRs. Conditioned-place preference (CPP) measures drug reward in mice. To determine whether TI-299423 was rewarding in mice, we asked if a low dose of TI-299423 (12 ng/Kg) could induce CPP in WT or α6L9'S* nAChR mice. Unexpectedly, TI-299423 induced significant CPP in WT, but not α6L9'S* mice, suggesting that TI-299423 had a more potent rewarding effect on WT mice than α6L9'S* mice. Measurements of TI-299423-induced [3H]dopamine release from midbrain synaptosomes confirmed that TI-299423 elicits more MII-

conotoxin-resistant (non-α6-mediated) , than MII-conotoxin-sensitive (α6-mediated), release from synaptosomes a this dose, suggesting that TI-299423 activation of the non-α6*nAChRs on dopaminergic neurons mediates its potent rewarding effects in WT mice. TI-299423 also potently and robustly activates α4β2 nAChRs expressed in N2A cells.

Disclosures: T. Wall: None. **B.N. Cohen:** None. **C.R. Wageman:** None. **S.R. Grady:** None. **M.J. Marks:** None. **B.H. Henderson:** None. **S. Mckinney:** None. **P. Deshpande:** None. **D. Yohannes:** A. Employment/Salary (full or part-time):; Targacept, Inc.. **H.A. Lester:** None.

Poster

225. **Catecholamine Receptors**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 225.01/D13

Topic: B.03. G-Protein Linked Receptors

Support: NIH-NIDA-IRP

Title: Looking for the molecular interface of dopamine D1-D3 receptor heteromers

Authors: *X. GUITART[1], G. NAVARRO[2], N.-S. CAI[3], E. MORENO[4], M. SANCHEZ[3], J. MALLOL[5], E. CANELA[5], C. LLUIS[5], A. CORTES[5], V. CASADO[5], P. J. MCCORMICK[5], S. FERRE[3];
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Abstract: Receptor heteromers are becoming the focus of extensive research in the field of G-protein-coupled receptors. Recent studies have shown evidence for the existence of heteromers between dopamine D_1 and D_3 receptors, which could have implications for basal ganglia disorders and drug addiction. In the present study we wanted to address the molecular interactions involved in D_1-D_3 receptor heteromerization, in particular, the role of transmembrane (TM) domains, by using synthetic peptides containing the amino acid sequence of TM domains of the D_1 receptor. Stably transfected cell lines (HEK293) expressing either the D_1 receptor fused to Rluc or the D_3 receptor fused to YFP or both were created. BRET analysis in D_1Rluc-D_3YFP cells (using D_1Rluc cells as controls) provided evidence for D_1-D_3 heteromerization. Unfortunately, and at odds with previously published studies, BRET analysis could not be used to study a possible destabilazing effect of TM peptides on D_1-D_3 heteromerization, due to a strong functional interference with Rluc. Nevertheless, using transient transfection of D_1 and D_3 receptors fused to potentially complementing moieties of YFP, TM5

and TM6 but not TM7 peptides were able to significantly decrease YFP-induced fluorescence. Importantly, TM5 and TM6 but not TM7 peptides counteracted D_1-D_3 cross-talk and cross-antagonism at the level of MAPK signaling in D_1Rluc-D_3YFP cells. These results indicate the selective involvement of TM5 and TM6 of D_1 receptor in the intermolecular and functional interactions between D_1 and D_3 receptors in the D_1-D_3 receptor heteromer. Looking for receptor-heteromer selective compounds, we also analyzed the ability of TM5 and TM6 peptides to selectively counteract ligand binding properties of D_3 receptor that depend on D_1-D_3 receptor heteromerization. This type of studies could clarify the role of the overexpression of the D_3 receptor described in several neuropsychiatric disorders, including drug addiction.

Disclosures: X. Guitart: None. **G. Navarro:** None. **N. Cai:** None. **E. Moreno:** None. **M. sanchez:** None. **J. Mallol:** None. **E. Canela:** None. **C. Lluis:** None. **A. Cortes:** None. **V. Casado:** None. **P.J. McCormick:** None. **S. Ferre:** None.

Poster

225. **Catecholamine Receptors**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 225.02/D14

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant NS047198

Title: Varying agonist efficacies of novel dopamine D3-selective ligands studied in the GTP-shift radioligand binding assay

Authors: J. ZHEN[1], M. E. A. REITH[1,2], *A. K. DUTTA, Dr[3];
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Abstract: Dopamine (DA) D2 and D3 receptors belong to the superfamily of G-protein-coupled-receptors (GPCRs). Agonist binding introduces conformational changes, compacting the extracellular agonist binding pocket and collapsing the central binding site of sodium. This provides a structural context for the well-known allosteric effect of Na^+ and GTP in shifting agonist potency in inhibiting the binding of a labeled antagonist towards lower affinity (GTP-shift assay). We hypothesized that this phenomenon represents the capability of agonists to convert and stabilize the intermediate or active states of DA D_2 or D_3 receptors, which is correlated with their potencies and efficacies to activate G protein in functional assays. The current study focused on the full agonists DA, quinpirole, and D-296, and on the partial agonists with varying efficacies (-)3PPP, D-301, D-303, and D-313 (for our novel D_3-selective D-ligands

aimed against PD see Biorg. Med. Chem. 2009: 17, 3923 and 2010: 18, 5661; J. Med. Chem. 2010: 53, 1023). Their potencies in inhibiting the binding of [^{3}H]spiperone to DA D_2 or D_3 receptors stably expressed in HEK293 and CHO cells were assessed under two different assay conditions (Mg^{2+} only – giving $K_{i\text{-}Mg}$, versus Na^+ and GTP_YS – giving $K_{i\text{-}Na}$). There was no difference between the two cell lines in the $K_{i\text{-}Na}$ values, but the $K_{i\text{-}Mg}$ values were significantly increased in CHO-D_3R ($P<0.001$). The affinity ratio $K_{i\text{-}Na}/K_{i\text{-}Mg}$, expressed as % of the ratio for DA, was strongly correlated between HEK-D_3R and CHO-D_3R ($Y=0.97*X+0.21$, $R^2=0.88$, $P<0.005$). This suggests that G protein coupling helped stabilize the active receptors (in absolute terms more in CHO-D_3R than in HEK- D_3R), but was not required for receptor activation. Thus, significant agonist-stimulated GTPγ^{35}S binding to cell membranes was not detected in HEK-D_3R. Interestingly, for both DA D_2 and D_3 Rs, $K_{i\text{-}Mg}$ in the [^{3}H]spiperone binding assay was highly correlated with EC_{50} in the functional [^{35}S]GTP_YS binding assay ($Y=1.05*X-0.03$, $R^2=0.91$, $P<0.0001$); the relative affinity ratio was also strongly linked with efficacy ($Y=0.80*X+0.27$, $R^2=0.68$, $P<0.01$). Thus, the capability of agonists to promote a more active state of DA D_2 or D_3 receptors is correlated with both their potency and efficacy to activate G protein. Additionally, the present results indicate that GTP-shift binding assays can be used for approximating partial D_2 and D_3 agonist properties.

Disclosures: J. Zhen: None. **A.K. Dutta:** None. **M.E.A. Reith:** None.

Poster

225. **Catecholamine Receptors**

Location: Halls B-H

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Program#/Poster#: 225.03/D15

Topic: B.03. G-Protein Linked Receptors

Support: NIMH Grant 5T32MH016434-33

Title: Evidence for limited D1 and D2 receptor co-expression and co-localization within striatal patch neurons of the neonatal mouse

Authors: *D. BIEZONSKI, P. TRIFILIEFF, J. JAVITCH, C. KELLENDONK;
Columbia Univ., New York, NY

Abstract: The striatum is the major input nucleus of the basal ganglia involved in reward processing, goal-directed behaviors, habit learning, and motor control. The striatum projects to the basal ganglia output nuclei via the “direct” and “indirect” pathways which can be distinguished on the basis of their projection fields and their opposing effects on behavior. In adult animals, the functional opposition is modulated by the differential actions of D1 and D2

dopamine receptors (D1R, D2R) whose expressions are largely separated between these pathways. Although extensively studied in the adult, little research exists on the relative expression patterns of D1 and D2 receptors early post-natally. To address this issue, we have used dual-label immunohistochemistry to map striatal D1 and D2R expression at the promoter level in post-natal day 1 (PD1) Drd1a-tdTomato/Drd2-eGFP BAC transgenic mice, and at the receptor level by co-staining for native D1 and D2Rs in wild-type P1 animals. We confirmed our observations with a recently developed proximity-ligation assay. In contrast to the limited overlap of both receptors in adults, our results demonstrate that shortly after birth some D1 and D2 receptors are co-expressed in the same neurons of the striatal patch - but not the matrix - compartment, the matrix exhibiting low levels of D1R expression. Moreover, we found that D1 and D2R proteins co-localize at a distance less than 16 nm suggesting that these receptors may have the potential to heterodimerize early post-natally. These novel findings pave the way for future research on the role dopamine receptors may play in early striatal ontogeny and function, and the significance this may have for proper development of striatally-mediated behaviors.

Disclosures: D. Biezonski: None. **C. Kellendonk:** None. **P. Trifilieff:** None. **J. Javitch:** None.

Poster

225. **Catecholamine Receptors**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 225.04/D16

Topic: B.03. G-Protein Linked Receptors

Support: NIH Intramural Program

NIH Grant R21 NS0676421

Title: Discovery and characterization of a completely G-protein biased D_2 dopamine receptor agonist

Authors: R. B. FREE[1], L. CHUN[1], J. CONROY[1], A. MORITZ[1], B. MILLER[1], T. DOYLE[1], A. PADRON[1], Y. HAN[2], N. SOUTHALL[3], J. XIAO[3], J. MARUGAN[3], M. FERRER[3], J. JAVITCH[2], *D. R. SIBLEY[1];
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Abstract: The D_2 dopamine receptor (DAR) is a highly validated drug target in neurology and psychiatry. For instance, receptor-based antiparkinsonian drugs primarily work via stimulating the D_2 DAR. However, most drugs targeting the D_2 DAR are problematic as they are either less

efficacious than desired or possess adverse side effects due to either cross reactivity or activation of inappropriate signaling pathways. A novel approach for attaining greater selectivity of therapeutic agents targeting the D_2 DAR is to identify and develop ligands that exhibit functional selectivity, or biased agonism. In contrast to most agonists, which activate all signaling pathways in parallel with equal efficacy, functionally biased agonists favor one transduction pathway over another. Here we report the discovery of a novel D_2 DAR agonist that is fully biased for G-protein-linked signaling while having no ability to activate β-arrestin-based signaling. We have also generated preliminary structure-activity relationship (SAR) data for this compound. We initially performed a high throughput-screening (HTS) campaign to interrogate a 380,000+ small molecule library to identify novel D_2 DAR agonists. The primary HTS assay utilized a cell line expressing the D_2 DAR coupled to a chimeric Gqi5 protein, thereby linking receptor activation to robust Ca^{2+} mobilization. We also conducted secondary assays to measure orthogonal D_2 DAR signaling activities including cAMP modulation and β-arrestin recruitment. The primary HTS screen resulted in the identification of 2,288 compounds with agonist activity. While the majority of the subsequently confirmed agonist hits activated all signaling pathways tested, some compounds showed a diminished ability to stimulate β-arrestin recruitment. One such compound, reported here, (MLS1547) is a full agonist at D_2 DAR mediated G-protein-linked signaling (Ca^{2+} and cAMP), but demonstrates no ability to activate β-arrestin recruitment (enzyme-complementation as well as BRET-based assays). The compound does, however, antagonize dopamine-stimulated β-arrestin recruitment to the D_2 DAR. Furthermore, the compound does not stimulate β-arrestin recruitment from any other DAR subtype (D_1-D_5). In an effort to further interrogate the chemical scaffold and identify a preliminary SAR that imparts this unique pharmacological profile; 20 analogs of MLS1547 were obtained or synthesized and analyzed in our assays. Interestingly, some of these analogs gain the ability to stimulate β-arrestin recruitment without losing the G-protein activating response. In summary, we have identified the first known compound that is a G-protein biased agonist of the D_2 DAR.

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Poster

225. **Catecholamine Receptors**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 225.05/D17

Topic: B.03. G-Protein Linked Receptors

Support: NIMH Grant R15MH091639

Rhode Island Institutional Development Award (IDeA) Network of Biomedical Research Excellence (INBRE) Award P20RR016457–10

Title: Functional recruitment of g protein beta5 to d2 dopamine receptors

Authors: *J. OCTEAU[1], J. CELVER[1], M. SHARMA[1], J. SCHRADER[1], C. COLVIN[1], C. AIUDI[2], K. SUN[2], C.-K. CHEN[3], A. KOVOOR[1];
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Abstract: G beta 5 (Gbeta5) is a G protein subunit that is thought to exist as obligate heterodimer with R7 RGS proteins. We found that while the majority of Gβ5 found in the cortex segregates into the detergent-soluble biochemical fraction, the majority of Gβ5 expressed in the striatum, a brain region that expresses high concentrations of D2R, segregates instead into the detergent-resistant biochemical fraction. We have previously shown that the vast majority of D2R either expressed endogenously in the brain or exogenously in cell lines segregates into detergent-resistant membrane (DRM) fractions. Therefore we asked if the detergent-insolubility of striatal Gβ5 was produced as a result of an interaction with D2R. When expressed in HEK293 cells Gβ5 is predominantly detergent-soluble but is re-targeted to the DRM after D2R co-expression. Furthermore, an in-cell proximity biotin-transfer assay indicated that D2R and Gβ5 interact in DRM and coexpression of D2R enhances the both the expression levels and stability of Gβ5. Gβ5 coexpression suppressed phosphorylation of MAP kinase (MAPK) elicited by dopamine-bound D2R and also altered the effects of D2R expression in a cellular adherence assay. Our data suggest that Gβ5 can exist as a complex with D2R in DRM independently of R7 RGS proteins since HEK293 cells do not endogenously express these proteins.

Disclosures: J. Octeau: None. **J. Celver:** None. **M. Sharma:** None. **J. Schrader:** None. **C. Colvin:** None. **C. Aiudi:** None. **K. Sun:** None. **A. Kovoor:** None. **C. Chen:** None.

Poster

225. Catecholamine Receptors

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 225.06/D18

Topic: B.03. G-Protein Linked Receptors

Support: MH093672

T32DA016224

Title: D2 receptor overexpression in accumbal indirect pathway medium spiny neurons leads to hyperexcitability and alterations in behavioral sensitization to cocaine

Authors: *E. F. GALLO[1], B. FENG[1], J. A. MORON CONCEPCION[2], J. A. JAVITCH[1], C. KELLENDONK[1];
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Abstract: The nucleus accumbens (NAc) is implicated in the neuroadaptations underlying drug addiction. Medium spiny neurons (MSNs), the main cellular output of the NAc, typically belong to either the direct or indirect projection pathways, which differ in their respective enrichment of D1 and D2 receptors (D1Rs and D2Rs). D2Rs play a key role in drug reinforcement, as demonstrated with pharmacological and gene knockout approaches. Moreover, neuroimaging studies indicate that chronic drug use in humans is associated with decreased D2R availability in the striatum. However, it is unknown whether selectively increasing D2R levels in the indirect pathway protects against the reinforcing effects of drugs of abuse, and if so, through which cellular mechanisms. Our previous work indicates that transgenic mice that overexpress striatal D2R (D2R-OE) since early development display increased MSN excitability, yet the consequences of inducing D2R overexpression in adult MSNs on excitability and drug-related behaviors is unknown.

To address these questions, we restricted D2R overexpression to indirect D2-expressing MSNs (D2-MSNs) by injecting a conditional adeno-associated virus encoding D2R into the NAc of Drd2-Cre transgenic mice. Using whole-cell patch clamp recordings in acute slices, we found that D2-MSNs overexpressing D2Rs show increased excitability compared to MSNs expressing EGFP. We also found a reduction in inwardly-rectifying K+ channel (Kir) currents, suggesting that, as in D2R-OE mice, D2R upregulation in adult MSNs may lead to hyperexcitability through downregulation of Kir function.

To begin to examine the behavioral effects of indirect pathway-specific D2R upregulation, we tested whether Drd2-Cre mice overexpressing D2R display alterations in cocaine-induced locomotor sensitization. We found that while D2R-overexpressing mice have a significantly higher basal locomotor activity compared to EGFP-expressing controls, both groups display a similar relative response to the first cocaine injection (15mg/kg, i.p.). However, unlike EGFP-expressing mice which progressively developed sensitization to daily cocaine injections, mice overexpressing D2R maintained the same locomotor response following repeated cocaine exposure. Together, these results suggest that indirect pathway-specific D2R upregulation in adult NAc leads to increased MSN excitability, which may be related to the observed alterations in locomotor activity and cocaine sensitization.

Disclosures: E.F. Gallo: None. **B. Feng:** None. **J.A. Moron Concepcion:** None. **J.A. Javitch:** None. **C. Kellendonk:** None.

Poster

225. **Catecholamine Receptors**

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Program#/Poster#: 225.07/D19

Topic: B.03. G-Protein Linked Receptors

Support: NIH grant NS058850

Title: Presynaptic dopamine D1 receptor regulation of the striatonigral GABA transmission

Authors: S. DING, *F.-M. ZHOU;
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Abstract: The GABAergic projection neurons (GABA neurons) are the main cell type in the substantia nigra pars reticulata (SNr), a key basal ganglia output nucleus that influences the thalamocortical motor circuit. The high frequency spontaneous firing in these GABA neurons is sculpted by excitatory and inhibitory synaptic inputs. SNr GABA neurons receive a robust GABA input from the direct pathway medium spiny neurons in the striatum (striatonigral pathway). Dopamine D1 receptors are abundantly expressed on the striatonigral axon terminals. Activated by endogenously released dopamine from the dendrites of nigral dopamine neurons, these D1 receptors may play an important role in regulating the striatonigral pathway. We hypothesize that D1 receptors facilitate the striatonigral GABA transmission at the axon terminal level and consequently reduces the output from the SNr. To test this idea, we have performed preliminary experiments in an angular sagittal brain slice preparation that enables the activation of the striatonigral projection without any apparent contamination from antidromically activated pallidonigral neurons. To avoid activating the potential presynaptic D2 receptors on other GABA afferents, we use the selective D1-like full agonist SKF81297. Our preliminary results indicate that SKF81297 facilitates striatonigral IPSCs in SNr GABA neurons. This facilitatory effect is blocked by the D1 receptor antagonist SKF83566. SKF81297 also increases the frequency but not the amplitude of miniature IPSCs in SNr GABA neurons. Taken together, these results indicate that dopamine D1 receptors on the striatonigral axon terminals promote the transmission of the inhibitory signal from the direct pathway medium spiny neurons to SNr GABA neurons. This facilitation may be important in normal movement control and also in Parkinson's disease.

Disclosures: S. Ding: None. **F. Zhou:** None.

Poster

225. **Catecholamine Receptors**

Location: Halls B-H

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Program#/Poster#: 225.08/D20

Topic: B.03. G-Protein Linked Receptors

Support: FONDECYT Nº 1110352

MSI N° P10-063-F

CONICYT doctoral thesis fellowship 24121352

ERC-250349

Title: Haloperidol-induced Nur77 expression depends on Thr34 DARPP-32 phosphorylation in striatopallidal medium spiny neurons of mice

Authors: *N. SANCHEZ[1], R. COURA[2], O. ENGMANN[2], L. MARION-POLL[2], S. LONGUEVILLE[2], D. HERVÉ[2], J.-A. GIRAULT[2], M. E. ANDRES[1];
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Abstract: Unbalanced signaling in striatopallidal and striatonigral striatal neurons impairs movements and may participate in diseases such as addiction and schizophrenia. Nur77 is an orphan nuclear receptor closely associated to dopamine signaling in these neurons. Classical antipsychotic drugs, such as haloperidol, are known to increase Nur77 expression in the striatum. However, little is known about the neuronal population in which Nur77 is induced by haloperidol and the intracellular signaling pathways involved. The striatal projecting GABA medium spiny neurons (MSN) are characterized by the segregated expression of D1 (striatonigral pathway) or D2 (striatopallidal pathway) dopamine receptors. Here, using pharmacological approaches and a variety of transgenic mice models, we investigated the mechanisms underlying the upregulation of Nur77 protein expression in the dorsal striatum after haloperidol injection. Our data show that Nur77 upregulation induced by haloperidol occurs preferentially in the D2 striatopallidal (MSN) cells and depends on Thr34 DARPP-32 phosphorylation. In addition, we showed that haloperidol-dependent Nur77 upregulation is mediated by A_{2a} signaling. Understanding the regulatory mechanisms of Nur77 expression in the striatum may help deciphering the molecular mechanisms underlying the extrapyramidal symptoms produced by haloperidol treatment.

Disclosures: N. Sanchez: None. **R. Coura:** None. **O. Engmann:** None. **L. Marion-Poll:** None. **S. Longueville:** None. **D. Hervé:** None. **J. Girault:** None. **M.E. Andres:** None.

Poster

225. **Catecholamine Receptors**

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Program#/Poster#: 225.09/D21

Topic: B.03. G-Protein Linked Receptors

Support: DC011080

Title: Cellular mechanisms for dopaminergic modulation of axon initial segment calcium channels

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Abstract: The axon initial segment (AIS) is a specialized neuronal subcompartment that supports action potential initiation. Recently, we found that low-voltage activated calcium channels enriched at the AIS are regulated by type 3 dopamine receptors (D_3R), and that this modulation strongly suppresses high frequency spike burst output. The cellular mechanisms by which dopamine modulates AIS calcium channels, and the identity of the channels modulated, has been unclear. We utilized 2-photon calcium imaging and electrophysiology in acute slice and cell culture preparations to probe the molecular pathway that underlies modulation of AIS calcium. In contrast to traditional G-protein mediated modulation, we found that D_3R signaling requires beta-arrestin and ERK1/2 to downregulate AIS calcium, suggesting that modulation of AIS channels is a novel example of biased signal transduction in neurons.
In previous experiments in dorsal cochlear nucleus cartwheel cells, we found that spike-evoked AIS calcium transients were sensitive to nickel and ascorbate, suggesting that the T-type isoform $Ca_V3.2$ is localized to the AIS. Consistent with these results, D_3R activation did not modulate residual AIS calcium transients in $Ca_V3.2$ knockout mice. Because neuronal voltage clamp of T-type currents is dominated by dendritic currents (Bender et al., 2011), the manner in which AIS $Ca_V3.2$ channels are modulated remains unknown. We therefore have transfected HEK cells with D_3R and $Ca_V3.2$ constructs. In isolated cells, D_3R activation reduced peak calcium currents 30 ± 1.5% (voltage steps from -100 to -40 mV, n = 8). We are currently utilizing this system to determine both the activity dependence of this modulation by D_3Rs and the molecular determinants in the receptor necessary for arrestin-dependent modulation.

Disclosures: S. Yang: None. **R.M. van Rijn:** None. **J.L. Whistler:** None. **K.J. Bender:** None.

Poster

225. **Catecholamine Receptors**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 225.10/D22

Topic: B.03. G-Protein Linked Receptors

Support: Estonian Science Foundation GARFS 8414

Estonian Ministry of Science and Education SF0180125s08

Title: Impaired dopamine signaling in dorsal striatum of Wfs1 deficient mice

Authors: *A. TERASMAA, T. VISNAPUU, C. A. HUNDAHL, E. VASAR;
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Abstract: Loss of function of the Wolframin (Wfs1) gene causes Wolfram syndrome, a rare multisystem degenerative disorder. Wfs1 gene is widely expressed in the brain and our previous work demonstrated that lack of Wfs1 protein leads to impaired function of dopaminergic system. Thus Wfs1 knock-out animals show diminished amphetamine induced locomotion. Also, the homozygous Wfs1 mutant mice show significantly
decreased striatal dopamine output in response to high-concentration K^+ challenge. The aim of this study is to further characterize the function of intracellular signaling in dopaminoceptive neurons of Wfs1-deficient mice. Acute administration of direct dopamine agonist apomorphine (3mg/kg, i.p.) induced an increase (163±11 % of baseline, $p<0.05$, n=5) in phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) at Thr34 in dorsal striatum of the wild type mice. Such effect of apomorphine was absent in Wfs1 knock-out mice (102±9 % of baseline, n=5). These results suggest that intracellular signaling downstream of dopamine receptor is altered in the dorsal striatum of Wfs1 deficient mice.

Disclosures: A. Terasmaa: None. **T. Visnapuu:** None. **C.A. Hundahl:** None. **E. Vasar:** None.

Poster

225. **Catecholamine Receptors**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 225.11/D23

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant R01 AG19230

Title: Modulation of canonical dopamine signaling system in hippocampus via formation of ghrelin and dopamine 1 receptor heteromers

Authors: *A. KERN, C. ULLRICH, R. ALBARRAN-ZECKLER, R. G. SMITH;
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Abstract: The orphan growth hormone secretagogue receptor (GHSR1a) first identified by reverse pharmacology and was subsequently de-orphanized by the discovery of the ghrelin. This cognate receptor for ghrelin belongs to the class A family of GPCRs. In the brain, GHSR1a is expressed mainly in hippocampal structures, hypothalamus and midbrain. Paradoxically, despite the broad expression of GHSR1a, other than trace amounts in the hypothalamus, endogenous ghrelin is undetectable in the brain; hence, we asked what the function of apo-GHSR1a might be. Using GHSR-IRES-GFP mice we identified subsets of neurons in the hippocampus that coexpress GHSR1a and DRD1. We speculated that apo-GHSR1a modifies DRD1 signaling. In a neuronal cell line coexpressing GHSR1a and DRD1, and in primary cultures of hippocampal neurons, we show that canonical DRD1 signaling is modified by GHSR1a such that dopamine (DA) treatment causes mobilization of intracellular Ca^{2+} ($[Ca^{2+}]_i$). Using pharmacological inhibitors, genetically encoded PLC and Ca^{2+} sensors, and BRET assays we dissected the signal transduction pathway. In the presence of GHSR1a, DRD1 couples to $G\alpha_q$ and DA activates the PLC and IP_3 signaling cascade. Furthermore, using Tr-FRET with SNAP and CLIP-tagged receptors, and inducible receptor homomerization assays, we found that non-canonical DA signaling is a consequence of formation of GHSR1a:DRD1 heteromers. The neutral GHSR1a antagonist (JMV2959) blocks DA-induced $[Ca^{2+}]_i$ release indicating that within the heteromer, one protomer allosterically modifies function of the other. The possibility of a mechanism involving receptor crosstalk, caused by basal activity of GHSR1a, was ruled out by experiments with: GHSR1a point mutants, selective Gα-protein knockdown, specific pharmacological inhibition of components of the $G\alpha_s$ signaling pathway. In mouse hippocampal brain slices we confirmed the presence of functional GHSR1a:DRD1 heteromers by FRET confocal microscopy and Ca^{2+} imaging. *In vivo* relevance was demonstrated by comparing DRD1 agonist-induced behavioral responses in mice treated with a neutral GHSR1a antagonist (JMV2959). The JMV2959 treated mice were resistant to DRD1 agonist-induced behaviors. Therefore, pharmacological intervention with a GHSR1a antagonist is a selective way to block DA signaling in neurons expressing GHSR1a:DRD1 heteromers, without affecting neurons expressing DRD1 alone. These results show the potential of designing new drugs for targeting psychiatric disorders associated with abnormal DA signaling by selectively targeting subsets of neurons expressing GHSR1a:DRD1.

Disclosures: A. Kern: None. **C. Ullrich:** None. **R. Albarran-Zeckler:** None. **R.G. Smith:** None.

Poster

225. Catecholamine Receptors

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Program#/Poster#: 225.12/D24

Topic: B.03. G-Protein Linked Receptors

Support: RR024158

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MH54137

DA026434

MH091360

Lieber Center for Schizophrenia Research

NIH NIDA IRP program

Title: Activation of G(s/olf) heterotrimers in living cells: Development of novel G(s/olf) biosensors

Authors: *H. YANO[1], D. PROVASI[2], M. FILIZOLA[2], S. FERRE[1], J. JAVITCH[3];
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Abstract: G protein-coupled receptors (GPCRs) mediate neurotransmission of numerous molecules. Their structure-function relationship is the key to better development of neuropharmacological agents. Conformational changes associated with GPCR activation have been revealed in remarkable details by the crystal structure of agonist-bound β2 adrenergic receptor (β2AR) coupled to G(s) as well as by complementary spectroscopy and imaging studies. However, the extent to which conformational changes in G protein are conserved in living cells as well as across different receptors and different G protein species, particularly G(olf), which shares 89% sequence homology, remains to be determined.
We first investigated conformational changes in the G(s) heterotrimer after activation of β2AR in intact cells. Using bioluminescence resonance energy transfer, we made observations on movements both within the G protein as well as between the receptor and the G protein. Using a library of novel G(s) biosensors with either luciferase or GFP inserted at various positions throughout the structure, we studied conformational changes in cells and compared the results to

the crystal structures of the inactive and active conformations. We also studied conformational changes in G(s) protein induced by the D1 dopamine receptor and A2A adenosine receptor. Finally, taking advantage of the significant homology, G(olf) biosensor constructs were made at the same ten positions used for G(s) and subsequently validated for measuring ligand activation. Our analysis using these G(s) biosensors suggests that conformational changes within the G(s) heterotrimer are very similar for different G(s)-coupling receptors. Comparison between the G(s) and G(olf) sensor readouts gives us a perspective on possible differences in the regulation of activation. Using this set of G(s) and G(olf) biosensors, partiality of agonists can be studied in relation to structural changes and subsequent effector activation. Further, activation preference between G(s) and G(olf) can be addressed. Thus, these biosensors represent a novel pharmacological tool to study structure-function relationships.

Disclosures: H. Yano: None. **D. Provasi:** None. **M. Filizola:** None. **S. Ferre:** None. **J. Javitch:** None.

Poster

225. **Catecholamine Receptors**

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Program#/Poster#: 225.13/D25

Topic: B.03. G-Protein Linked Receptors

Support: CONACyT

Title: Differential role of α2-adrenoceptor subtypes in the inhibition of the vasopressor sympathetic outflow in diabetic pithed rats

Authors: *A. H. ALTAMIRANO-ESPINOZA, G. MANRIQUE-MALDONADO, I. I. RUIZ-SALINAS, B. VILLANUEVA-CASTILLO, C. M. VILLALÓN;
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Abstract: Several lines of evidence suggest a link between diabetes and disturbances on the modulation of blood pressure, related with pre/post-junctional α2-adrenoceptors on blood vessels from different species including humans. Hence, we hypothesize that the expression of different α2-adrenoceptor subtypes mediating sympatho-inhibition and/or systemic vasoconstriction (i.e. vasopressor responses) could be altered in diabetic subjects, probably as a compensatory mechanism. On this basis, the present study identified the pharmacological profile of the α2-adrenoceptor subtypes mediating inhibition of the sympathetic vasopressor outflow in diabetic pithed rats. For this purpose, 125 male Wistar rats (95 pretreated i.p. with 50 mg/kg streptozotocin and 30 pretreated i.p. with vehicle) were anaesthetized, pithed and artificially

respired with room air (56 strokes/min; stroke volume: 20 ml/kg). Under these conditions, electrical stimulation (0.03, 0.1, 0.3, 1 and 3 Hz; 50 V and 2 ms) of the thoracic (T7-T9) preganglionic sympathetic vasopressor outflow or i.v. bolus injections of exogenous noradrenaline (0.03, 0.1, 0.3, 1 and 3 μg/kg) resulted in frequency dependent (n=105) and dose-dependent (n=20) vasopressor responses, respectively. Furthermore, i.v. continuous infusions of the α2-adrenoceptor agonist, BHT 933 (10 and 30 μg/kg.min), inhibited the sympathetically-induced, but not the noradrenaline-induced, vasopressor responses. Interestingly, in euglycemic (vehicle-treated) rats, lower doses of BHT 9333 (1 and 3 μg/kg.min), which were inactive in diabetic rats, dose-dependently inhibited the sympathetic vasopressor outflow. Furthermore, after i.v. administration of selective antagonists, the BHT 933-induced sympatho-inhibition in diabetic rats was: (i) abolished after BRL-44408 (α2A; 100 μg/kg); (ii) dose-dependently, but not completely, blocked by imiloxan (α2B; 300 and 1000 μg/kg); and (iii) unchanged after JP 1302 (α2C; 1000 μg/kg). These doses of antagonists, which did not affect per se the sympathetically-induced vasopressor responses, were high enough to completely block their respective receptors. In conclusion, these results in diabetic rats suggest that the inhibition of the sympathetic vasopressor outflow produced by 10 μg/kg.min BHT 933 is predominantly mediated by the α2A- and, to a lesser extent, by the α2B-adrenoceptor subtypes, with no evidence for the role of the α2C subtype. This pharmacological profile contrasts with that of the α2 adrenoceptors mediating sympatho-inhibition in euglycemic rats, in which mainly α2A/2C- but not α2B-, adrenoceptors are involved.

Disclosures: A.H. Altamirano-Espinoza: None. **G. Manrique-Maldonado:** None. **B. Villanueva-Castillo:** None. **I.I. Ruiz-Salinas:** None. **C.M. Villalón:** None.

Poster

225. **Catecholamine Receptors**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 225.14/D26

Topic: B.03. G-Protein Linked Receptors

Title: Lasting activity of alpha1-AR during postnatal development alters membrane properties of LC in SHR

Authors: S. IGATA[1,2], T. HAYASHI[3], M. ITOH[2], M. TAKANO[2], *M. ISHIMATSU[4,2]; [1]Dept. Healt. Sci., Kyushu Univ., Fukuoka, Japan; [2]Dept. Physiol, [3]Urol, Kurume Univ. Sch. Med., Kurume, Japan; [4]Nishikyushu Univ., Kanzaki, Japan

Abstract: Attention Deficit Hyperactivity Disorder (ADHD) is a developmental disorder commonly found in childhood. Several lines of evidence indicate that locus coeruleus (LC) is the brain region involved in attention, vigilance, and cognitive functions, key clinical features of ADHD. Using a spontaneously hypertensive rat (SHR), a widely used ADHD animal model displaying ADHD like behaviors starting from 3 to 4 weeks old, we characterized synaptic transmission in LC neurons during the course of postnatal development. In particular, we analyzed membrane properties by whole cell patch clamp technique at two developmental time points, postnatal day (P) 3-5 (neonates) and P21-28 (juveniles). The data measured in SHR LC neurons were compared to those in age matched Wistar rats as normotensive controls. So far, our data showed no statistical changes in resting membrane potential (RMP) and spontaneous firing frequency (SFR) in the neonatal Wistar and SHR. However, we found age specific alterations in the juvenile SHR LC neurons; significantly depolarized RMP and significantly higher SFR. Noradrenaline is a prominent neurotransmitter in LC, and induces an outward current through an inwardly rectifier potassium channel coupled to alpha2-adrenergic receptor (AR) activation. Since previous study demonstrated the expression of functional alpha1-AR in the neonatal rats, we asked whether alpha1-AR contributes to altered membrane properties in the LC neurons of SHR in a postnatal development dependent manner. The neonatal LC neurons of both SHR and Wistar produced Phenylephrine (PE), an alpha1-AR-agonist, induced inward currents. In contrast, the juvenile LC neurons of SHR, but not Wistar, produced the PE induced inward currents, which were abolished by TRP channel blockers. Furthermore in the presence of yohimbine, application of an alpha1-AR antagonist, Prazosin, to the juvenile SHR LC neurons resulted in an outward current, indicating that basal activity of alpha1-AR modulates the resting membrane potential of the juvenile SHR LC neurons. Importantly, neither PE nor prazosin affected the RMP of the juvenile LC neurons of Wistar. These results indicates the loss of alpha1-AR function during normal rodent development. In contrast, the LC neurons of SHR persistently display the basal activity of alpha1-AR throughout the postnatal development, possibly leading to the depolarized membrane potential and increased spontaneous firing. In summary, our study highlights the alterations in the membrane properties in the LC neurons of SHR, which occur in the postnatal development dependent manner, may underlie pathophysiology of ADHD.

Disclosures: S. Igata: None. **T. Hayashi:** None. **M. Itoh:** None. **M. Takano:** None. **M. Ishimatsu:** None.

Poster

225. **Catecholamine Receptors**

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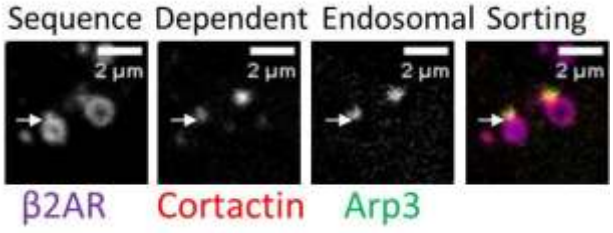
Topic: B.03. G-Protein Linked Receptors

Support: NIH Training Grant 2 R90 DA23420

Title: Heterologous signaling via Src regulates sequence-dependent recycling of signaling receptors via modification of cortactin

Authors: *R. VISTEIN, M. PUTHENVEEDU;
Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Post-endocytic recycling of neurotransmitter receptors from endosomes is a critical step that determines the resensitization of neurons to signals. While some proteins can recycle without apparent requirements, many signaling proteins recycle through a highly defined actin- and sequence-dependent pathway. Why signaling receptors have these additional requirements is not clear. Here, using high resolution Total internal reflection fluorescence (TIRF) and spinning disk confocal microscopy to visualize the recycling of the beta-2 adrenergic receptor (B2AR) in live cells, we show that B2AR recycling through the sequence-dependent pathway is regulated by the Src Kinase. This regulation is restricted to proteins that recycle via the sequence-dependent pathway. Src kinase regulates the recycling rate of B2AR via phosphorylation of cortactin, an actin nucleation promotion factor, which controls the rate of generation of vesicles in this pathway. Together, our results suggest that the restriction of signaling receptors to a sequence-dependent recycling pathway allows for heterologous signaling pathways to control receptor recycling and neuronal resensitization to signals.



Disclosures: R. Vistein: None. **M. Puthenveedu:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.01/D28

Topic: B.04. Ion Channels

Support: Grants from the Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs

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Title: A novel $Na_v1.8$ mutation in small fiber neuropathy: Impaired inactivation underlying DRG neuron hyperexcitability

Authors: *C. HAN[1,2], D. VASYLYEV[1,2], L. J. MACALA[1,2], M. M. GERRITS[3], J. G. J. HOEIJMAKERS[4], K. J. BEKELAAR[4], S. D. DIB-HAJJ[1,2], C. G. FABER[4], I. S. J. MERKIES[4,5], S. G. WAXMAN[1,2];
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Abstract: Painful small-fiber neuropathy represents a significant public health problem. In approximately one-half of cases (termed idiopathic) a cause cannot be identified. Gain-of-function mutations of the sodium channel $Na_V1.7$ have recently been identified in nearly 30% of patients with biopsy confirmed idiopathic small-fiber neuropathy. More recently, gain-of-function mutations of sodium channel $Na_V1.8$ have been found in a smaller percentage of patients with small-fiber neuropathy. Previously described mutations of $Na_V1.8$ associated with painful neuropathy accelerate recovery from inactivation (repriming), enhance the response to slow depolarizations, and enhance activation at the channel level, and produce hyperexcitability of small DRG neurons, which include nociceptors, at the cellular level. We have now identified a new $Na_V1.8$ mutation from two patients with painful small-fiber neuropathy, and our preliminary studies suggest a previously unreported mode of gain-of-function of $Na_V1.8$, i.e. impaired fast-inactivation. We also show that the mutant channel causes DRG neuron hyperexcitability. Together with our earlier results, our observations support the conclusion that an array of Nav1.8 mutations, which affect channel function in multiple ways, can contribute to the pathophysiology of painful peripheral neuropathy.

Disclosures: C. Han: None. **D. Vasylyev:** None. **L.J. Macala:** None. **M.M. Gerrits:** None. **J.G.J. Hoeijmakers:** None. **K.J. Bekelaar:** None. **S.D. Dib-Hajj:** None. **C.G. Faber:** None. **I.S.J. Merkies:** None. **S.G. Waxman:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: B.04. Ion Channels

Support: ARC Grant DP120102389

Title: The role of 'neonatal' form of $Na_V1.2$ in neuronal function and brain development

Authors: *E. GAZINA, B. LEAW, K. RICHARDS, C. REID, S. PETROU;
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Abstract: Four Na^+-channel α subunits are expressed at high levels in human brain. Each of them has a 'neonatal' and an 'adult' form due to developmentally regulated alternative splicing of coding exons 5N and 5A. This splicing event is largely conserved between humans and mice, suggesting that it has an important biological function which is currently unknown. The Na^+-channel α subunit $Na_V1.2$ undergoes the largest 'neonatal' to 'adult' transition in postnatal mouse cortex: from 2:1 'neonatal' to 'adult' ratio at birth to 1:13 at 15 days of age. When expressed in HEK293 cells, the 'neonatal' form of human $Na_V1.2$ is less excitable than the 'adult' form, suggesting that alternative splicing may regulate neuronal excitability during brain development. To examine the functional role of the 'neonatal' $Na_V1.2$, we mutated the 5N exon, thus creating a mouse line that expresses 'adult' $Na_V1.2$ regardless of the splicing pattern. Electrophysiological properties of L2/3 cortical pyramidal neurons of the wild-type and homozygous 'adult $Na_V1.2$' mice were then compared using whole-cell patch-clamp. At P3 the 'adult $Na_V1.2$' mice had an excitable phenotype with an increase in action potential firing. Furthermore, the 'adult $Na_V1.2$' cortices contained a population of fast-firing neurons (23% of total) that were not present in the wild-type mice. At P15 the action potential firing in neurons of the mutant and the wild-type mice became identical, consistent with the dominance of 'adult' $Na_V1.2$ in the wild-type cortex at this age. Nevertheless, adult mutant mice (P45-90) had a significantly reduced threshold for proconvulsant-induced seizures compared to the wild-type. They also exhibited behavioural differences from their wild-type siblings. For example, the 'adult $Na_V1.2$' mice spent significantly more time on the open arms of the elevated plus maze than the wild-type.
In summary, these results demonstrated that expression of the 'neonatal' $Na_V1.2$ reduces excitability of pyramidal cortical neurons in neonatal wild-type mice. Moreover, the expression of the 'neonatal' $Na_V1.2$ at young age has long-term effects on behaviour and seizure susceptibility, likely due to its role in development of neuronal networks.

Disclosures: E. Gazina: None. **B. Leaw:** None. **K. Richards:** None. **C. Reid:** None. **S. Petrou:** None.

Poster

226. **Ion Channels: Na+ Channels**

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Topic: B.04. Ion Channels

Support: Dept. of Veternas Affairs

R01 NS34509

Rackham School of Graduate Studies, University of Michigan

Title: Functional characterization of a novel Nav1.6 mutation in a patient with infantile epilepsy

Authors: *M. R. ESTACION[1,2], J. E. O'BRIEN[3], D. TALWAR[4], S. D. DIB-HAJJ[1,2], M. F. HAMMER[4], M. H. MEISLER[3], S. G. WAXMAN[1];
[1]Neurol, Yale Univ. Sch. of Med., New Haven, CT; [2]The Rehabil. Res. Ctr., VA Connecticut Healthcare Syst., West Haven, CT; [3]Dept. of Human Genet., Univ. of Michigan, Ann Arbor, MI; [4]Div. of Biotech., Univ. of Arizona, Tuscon, AZ

Abstract: The etiology of neurodevelopmental disorders such as epilepsy represent a considerable challenge for molecular genetic analysis because of marked genetic heterogeneity, but it has been hypothesized that rare or novel point mutations may contribute to a substantial number of these cases. When the observed phenotype is particularly severe and there is no prior family history of the disorder, it is reasonable to consider a disease model that involves a dominant de novo mutation. Support for this model comes from studies of epileptic encephalopathies, where de novo mutations of several genes have been observed, including many mutations in the voltage-gated sodium channel gene SCN1A in individuals with Dravet Syndrome (MIM 607208). The etiology for many cases of epileptic encephalopathy remains unknown, suggesting that there may be causal mutations in additional genes. Using a whole genome sequencing approach and electrophysiological recordings, we previously identified the first mutation of SCN8A (sodium channel Nav1.6) in a patient with infantile epileptic encephalopathy and SUDEP (Veeramah et al, AJHG 2012). The de novo mutation hNav1.6-N1768D resulted in a distinctive 5-fold increase of persistent current. Current-clamp analysis in hippocampal neurons transfected with N1768D channels revealed increased spontaneous firing, paroxysmal depolarizing shift (PDS)-like complexes, and increased firing frequency, consistent with a dominant gain-of-function phenotype in the heterozygous proband. We now report a second heterozygous de novo SCN8A mutation in another patient with infantile epilepsy. Using voltage-clamp recording in the neuronal cell line ND7/23 we observed a significant hyperpolarizing shift of the voltage-dependence of activation for the mutant channel as well as an enhancement of ramp current. Both properties are predicted to make neurons hyperexcitable, consistent with the clinical phenotype. Functional characterization of this second Nav1.6 channel

mutation provides further evidence supporting the inclusion of SCN8A as a causative gene in infantile epilepsy.

Disclosures: M.R. Estacion: None. **J.E. O'Brien:** None. **D. Talwar:** None. **S.D. Dib-Hajj:** None. **M.F. Hammer:** None. **M.H. Meisler:** None. **S.G. Waxman:** None.

Poster

226. **Ion Channels: Na+ Channels**

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Program#/Poster#: 226.04/D31

Topic: B.04. Ion Channels

Support: Israel Science Foundation grant 1593/10

Title: Inactivation properties of Na^+ channels in the axonal spike trigger zone of cortical pyramidal neurons

Authors: *I. A. FLEIDERVISH, G. BARANAUSKAS;
Dept. of Physiol., Ben Gurion Univ., Beer Sheva, Israel

Abstract: In cortical pyramidal cells, as in many CNS neurons, action potentials (APs) initiate in the axon initial segment (AIS) - the proximal part of the axon where the neuronal membrane is not covered with a myelin sheath and which possesses a distinctive, specialized assembly of voltage gated channels and associated proteins. Preferable AIS spike initiation is explained, at least partially, by lower activation voltage of local Na^+ channels. To what extent the inactivation properties of these channels differ from those in soma and dendrites? Molecular studies suggest that inactivation is voltage independent process that derives all of its apparent voltage-dependence from activation. Thus, hyperpolarizing shift in channel steady state activation characteristics should lead to an equivalent shift in its steady state inactivation curve. This theoretically expected shift, if it exists, could cause reduction in channels availability in the subthreshold range of voltages and, paradoxically, to render the AIS less excitable.
Here, we probed the voltage dependence of the axonal Na^+ channel inactivation by measuring changes in fluorescence of Na^+ -sensitive dye, SBFI, elicited by single AP in layer 5 pyramidal neurons in neocortical slices. As with conventional Na^+ current recordings, we assumed that the amplitude of Na^+ flux could provide us with an estimate of Na^+ channel availability before an AP was fired. We found that axonal Na^+ channel availability was nearly maximal at resting potential, as stepping the membrane even to very negative potentials caused no significant increase in Na^+ flux. By applying slow depolarizing current ramps to the soma, we found that availability of the AIS Na^+ channels changes very little (<20 %) by depolarization to voltages of up to -55 mV.

Our data indicate that voltage dependence of inactivation of the AIS Na^+ channels is rightward shifted as compared with somatic channels, making them fully available even at very depolarizing voltages.

Disclosures: I.A. Fleidervish: None. **G. Baranauskas:** None.

Poster

226. Ion Channels: Na+ Channels

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Program#/Poster#: 226.05/D32

Topic: B.04. Ion Channels

Support: National Basic Research Program of China (2010CB529806)

National Natural Science Foundation of China (31171064)

Key Research Program of Science and Technology Commissions of Shanghai Municipality (11JC1404300)

Leading Academic Discipline Project of Shanghai Municipal Education Commission (J50108)

Title: Exploring the obscure profiles of pharmacological binding sites on voltage-gated sodium channels by bmk neurotoxins

Authors: Z. LIU[1,2], *Y.-H. JI[1];
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Abstract: Diverse subtypes of voltage-gated sodium channels (VGSCs) have been found throughout tissues of the brain, muscles and the heart. Neurotoxins extracted from the venom of the Asian scorpion *Buthus martensi* Karsch (BmK) act as sodium channel-specific modulators and have therefore been widely used to study VGSCs. α-type neurotoxins, named BmK I, BmK αIV and BmK abT, bind to receptor site 3 on VGSCs and can strongly prolong the inactivation phase of VGSCs. In contrast, β-type neurotoxins, named BmK AS, BmK AS-1, BmK IT and BmK IT2, occupy receptor site 4 on VGSCs and can suppress peak currents and hyperpolarize the activation kinetics of sodium channels. Accumulating evidences from binding assays of scorpion neurotoxins on VGSCs however indicate that pharmacological sensitivity of VGSC subtypes to different modulators is much more complex than that suggested by the simple α-type and β-type neurotoxin distinction. Exploring the mechanisms of possible dynamic interactions

between site 3- / 4-specific modulators and region- and/or species-specific subtypes of VGSCs would therefore greatly expand our understanding of the physiological and pharmacological properties of diverse VGSCs. In this review, we discuss the pharmacological and structural diversity of VGSCs as revealed by studies exploring the binding properties and cross-competitive binding of site 3- or site 4-specific modulators on VGSC subtypes in synaptosomes from distinct tissues of diverse species.

Disclosures: Z. Liu: None. **Y. Ji:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.06/D33

Topic: B.04. Ion Channels

Title: Effect of selective Nav1.7 blockers on action potential generation of cultured mouse DRGs, human DRGs and hESC-derived sensory neurons

Authors: *E. B. STEVENS[1], A. R. BROWN[1], L. CAO[1], P.-P. SAINTOT[1], G. T. YOUNG[1], M. L. CHAPMAN[2], A. NITZSCHE[1], J. G. BILSLAND[1], R. P. BUTT[1], C. E. PAYNE[1];
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Abstract: Evidence from clinical pain genetics , transgenic mouse models and detailed channel biophysics suggest that Nav1.7 has a key role in action potential electrogenesis in sensory neurons (Dib-Hajj et al., 2013).
The specific role of Nav1.7 in action potential generation was studied using highly selective small molecule blockers of Nav1.7. Effects of Nav1.7 currents were measured in 3 different cultured sensory neuron models: small diameter mouse DRG neurons, small diameter human DRG neurons and human embryonic stem cell (hESC)-derived sensory neurones using whole-cell patch clamp recordings. We have previously demonstrated that Nav1.7 is the major component underlying the TTX-S current in mouse and human DRGs (Alexandrou et al., 2012), in contrast Nav1.7 constitutes approximately 30% of the TTX-S current in hESC-derived sensory neurons. Using current-clamp recordings single action potentials were evoked using current injections just above threshold for firing. Selective Nav1.7 blockers caused either a change in action potential waveform parameters or a complete ablation of action potential firing in all three sensory neuron preparations. In particular, there was a significant decrease in both rate of rise of the upstroke and amplitude of action potentials. These data provide further evidence that Nav1.7 has an important role in controlling sensory neuron action potential propagation through its

contribution to electrogenesis at threshold of firing.
Dib-Hajj S.D., Yang Y., Black J.A. & Waxman S.G. (2013) The Na(V)1.7 sodium channel: from molecule to man. Nat Rev Neurosci. 14:49-62.
Alexandrou AJ, Turner J, Payne L, Cox PJ, Panchenko VA, Ghetti A, Prime R, Doyle R, Chapman M, Marron B, Miller, PE, Butt RP, Stevens EB (2012) Nav1.7 channel is the dominant subtype underlying TTX-S currents in small diameter mouse DRGs. Program No. 140.05/C24. 2012 New Orleans, LA: Society for Neuroscience, 2012.

Disclosures: **E.B. Stevens:** A. Employment/Salary (full or part-time):; Pfizer. **A.R. Brown:** A. Employment/Salary (full or part-time):; Pfizer. **L. Cao:** A. Employment/Salary (full or part-time):; Pfizer. **P. Saintot:** A. Employment/Salary (full or part-time):; Pfizer. **G.T. Young:** A. Employment/Salary (full or part-time):; Pfizer. **J.G. Bilsland:** A. Employment/Salary (full or part-time):; Pfizer. **C.E. Payne:** A. Employment/Salary (full or part-time):; Pfizer. **R.P. Butt:** A. Employment/Salary (full or part-time):; Pfizer. **A. Nitzsche:** A. Employment/Salary (full or part-time):; Pfizer. **M.L. Chapman:** A. Employment/Salary (full or part-time):; Pfizer.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.07/D34

Topic: B.04. Ion Channels

Title: Analysis of inward excitatory currents during an action potential from suprachiasmatic nucleus neurons

Authors: *J. R. CLAY;
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Abstract: The ionic currents underlying excitability in suprachiasmatic nucleus (SCN) neurons have been analyzed in voltage clamp conditions using both voltage steps and the action potential (AP) clamp method (AC Jackson, GL Yao, BP Bean, J. Neurosci. 24:7985, 2004). In this study one of the APs in their report was digitized and applied to models of the excitatory sodium current, I_{Na}, and the calcium current, I_{Ca}, in voltage-clamp, i.e., a computational method of implementing the AP clamp technique. The Hodgkin-Huxley m^3h model was used in the I_{Na} analysis. Initially, rate parameters similar to theirs were also used. That analysis yielded a significant I_{Na} during AP repolarization, as occurs in squid giant axons but not in mammalian neurons. Using I_{Na} rate constants from an analysis of this component in hippocampal mossy fibers as the starting point for the SCN I_{Na} results was successful. The model predicts maximum

I_{Na} during the latter part of the upstroke of the AP followed by a rapid decline to near-zero during the remainder of the AP, as observed experimentally. A d^2f model was used in the I_{Ca} analysis. Initially, voltage-dependent inactivation was used for the f parameter. This was replaced by calcium current dependent inactivation. The model successfully describes I_{Ca} results obtained from SCN neurons both with voltage steps and action potential clamp. These results provide building blocks for a full model of the AP from SCN neurons. They also demonstrate a general method for building models of the AP for other excitable cells.

Disclosures: J.R. Clay: None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.08/D35

Topic: B.04. Ion Channels

Support: Pfizer Neusentis

Title: Inhibition of excitatory synaptic transmission in mouse spinal cord by a $Na_v1.7$ selective sodium channel antagonist

Authors: *M. K. PATEL[1], M. CHAPMAN[2], E. B. STEVENS[3], D. GRYDER[1];
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Abstract: In view of the importance of Na channels in cellular excitability, pharmacological modulation of their activity has proved beneficial in the treatment of many disorders including pain. In this study, we have examined the effects of a sodium channel antagonist selective for the Nav1.7 isoform (Compound A), on synaptic transmission onto substantia gelatinosa (SG) neurons of the mouse dorsal horn.
Spinal cord slices (350 μm) were superfused with solution comprising (mM) 125.0 NaCl, 25.0 $NaHCO_3$, 10.0 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $CaCl_2$, 1.0 $MgCl_2$ bubbled with 95%, 5% O_2/CO_2. Electrophysiological recordings were made from visually identified lamina II SG neurons using electrodes filled with (mM) 120.0 Kgluconate, 10.0 NaCl, 2.0 $MgCl_2$, 0.5 K_2EGTA, 10.0 HEPES, 4.0 Na_2ATP, 0.3 Na_2GTP, 5 μM QX314, pH 7.2 and held at –60 mV. Stimulation of the dorsal root using a suction electrode evoked both Aδ and C fiber excitatory post synaptic currents (EPSC). All EPSC's were recorded in the presence of 50 μM picrotoxin and strychnine. Values are mean ± SE. All animal experiments were approved by the Institute of Animal Care and Use Committee.

Evoked EPSC's were completely abolished by NBQX (5μM) and TTX (500 nM). Bath application of Compound A at 30 nM (20-30 mins) inhibited Aδ fiber responses by 46.9 ± 5.4% (n=4) and C fiber responses by 53.7 ± 10.1% (n=8). At 100 nM Compound A, the effects on EPSC's were more pronounced. Aδ fiber responses were inhibited by 90.2 ± 7.0% (n=5) while C fiber responses were inhibited by 88.2 ± 5.9% (n=10). Prolonged washout (>30 mins) resulted in only partial recovery of EPSC amplitudes in a few neurons. Under current clamp conditions, stimulation of the dorsal root evoked a single action potential from SG neurons. In the presence of 30 nM Compound A, evoked action potentials were abolished in 7 out of 13 neurons tested. The effects of Compound A were compared with the clinically available sodium channel antagonist mexilitine (100μM). Mexilitine reduced Aδ fiber amplitudes by 71.8 ± 13.9% (n=5) and C fiber responses by 83.4 ± 14.5% (n=5). In summary, we have shown that Compound A, a sodium channel antagonist with selectivity against Nav1.7, is capable of modulating excitatory synaptic transmission within the dorsal horn.

Disclosures: M.K. Patel: None. **M. Chapman:** A. Employment/Salary (full or part-time):; Neusentis. **E.B. Stevens:** A. Employment/Salary (full or part-time):; Pfizer Neusentis. **D. Gryder:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.09/D36

Topic: B.04. Ion Channels

Support: NIAAA 1R01AA020501

INIA

Title: Sodium channel β4 auxiliary subunit (Scn4b) controls spike-timing-dependent plasticity in core nucleus accumbens medium spiny neurons

Authors: X. JI[1], *G. E. MARTIN[2];
[1]Psychiatry, UMass Med. Sch., Worcester, MA; [2]UMass Med. Sch., WORCESTER, MA

Abstract: Alcoholism is the most widespread and costly form of drug addiction in the world. A number of studies have identified genes whose expression is associated with a greater propensity to drink alcohol in mice and rats. However, there is currently very little information regarding their functional role in brain regions that are directly responsible for dependence to the drug. In the core nucleus accumbens, known for its key role in drug addiction and reward, we

investigated the role of Scn4b, one of the four currently known sodium channel auxiliary subunits. Recent works reported that Scn4b expression is correlated with high alcohol-drinking intake in mice and rats. In a previous study in the same brain region, we explored spike-timing-dependent plasticity (STDP), a stimulation paradigm widely used to study synaptic plasticity and to evoke both long-term potentiation (tLTP) and long-term depression (tLTD). Surprisingly, we showed that tLTP depended on NMDA receptors, while tLTD relied on action potentials only. Indeed, blockade or alterations of action potential properties selectively and robustly inhibited LTD, but left LTP intact. Therefore, we hypothesized that alterations of Scn4b expression similarly and specifically targeted tLTD. In C57Bl/6 mice accumbens medium spiny neurons, we knocked down Scn4b expression using lentivirus Scn4b shRNA injected bilaterally. Two weeks after injections, we compared synaptic plasticity in these mice with those injected with the virus only, or with control (non-injected) animals. We found that injection of the virus alone had little effects on both tLTP and tLTD as amplitude and ratio were similar to that of control mice. In contrast, in neurons transfected with shRNA, tLTD amplitude was strongly reduced. Importantly, we showed that the ratio tLTP/tLTD was markedly shifted towards tLTP. Our study shows for the first time the selective role of sodium channel auxiliary subunit in synaptic plasticity and tLTD. Additional studies are needed to further establish the relationship between Scn4b expression, tLTD, and excessive drinking.

Disclosures: X. Ji: None. **G.E. Martin:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.10/D37

Topic: B.04. Ion Channels

Title: Nav1.7 inhibitory peptide from tarantula venom

Authors: *B. D. MOYER[1], D. LIU[1], J. LIGUTTI[1], A. ZOU[1], J. K. MURRAY[2], L. P. MIRANDA[2], S. I. MCDONOUGH[3];
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Abstract: Peptides isolated from animal venom have been useful tools for determining ion channel function, structure, and gating. In screening for novel inhibitors of the voltage-gated sodium channel Nav1.7, venom collected from the Chilean rose tarantula (*Grammastola porteri*) was fractionated and an active peptide, termed GpTx-1, was identified. GpTx-1 is a member of the inhibitory cysteine knot family of peptide toxins that includes HwTx-IV and ProTx-II.

Characterized with patch-clamp electrophysiology, GpTx-1 rapidly and reversibly inhibited human Nav1.7 with an IC50 ~ 10 nM. GpTx-1 inhibition was partially relieved by strong depolarizations delivered at high frequency, suggesting that GpTx-1 binding reduces Nav1.7 sensitivity to voltage and that GpTx-1 has lower affinity for channel open states. GpTx-1 was not more potent when tested on channels with 20% fractional inactivation, suggesting most prominent binding to closed channels. GpTx-1 also inhibited human Nav1.3 (IC50 ~ 40 nM) and human Nav1.4 (IC50 ~200 nM) but was more selective over human Nav1.5 (IC50 > 5 uM). GpTx-1 rapidly and reversibly inhibited endogenous tetrodotoxin-sensitive (TTX-S) sodium channel currents recorded from acutely dissociated mouse dorsal root ganglion sensory neurons with an IC50 ~ 5 nM. In summary, GpTx-1 peptide, identified from tarantula venom, is a potent human Nav1.7 inhibitor that is selective over the cardiac (Nav1.5) Nav isoform.

Disclosures: B.D. Moyer: A. Employment/Salary (full or part-time):; Amgen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen. **D. Liu:** A. Employment/Salary (full or part-time):; Amgen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen. **J. Ligutti:** A. Employment/Salary (full or part-time):; Amgen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen. **A. Zou:** A. Employment/Salary (full or part-time):; Amgen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen. **J.K. Murray:** A. Employment/Salary (full or part-time):; Amgen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen. **L.P. Miranda:** A. Employment/Salary (full or part-time):; Amgen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen. **S.I. McDonough:** A. Employment/Salary (full or part-time):; Amgen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.11/D38

Topic: B.04. Ion Channels

Support: NIH Grant GM48677

Title: Co-expression of β-subunits with α-subunits modify the conotoxin-susceptibilities of voltage-gated sodium channels

Authors: M.-M. ZHANG[1], L. AZAM[1], J. GAJEWIAK[1], J. IMPERIAL[1], G. BULAJ[2], B. M. OLIVERA[1], *D. YOSHIKAMI[1];
[1]Biol., [2]Medicinal Chem., Univ. Utah, Salt Lake City, UT

Abstract: Four families of conotoxins have been identified thus far that target voltage-gated sodium channels. Each family has a distinct mechanism of action: μ-conotoxins are pore blockers, μO-conotoxins block channel activation, ι-conotoxins promote channel activation, and δ-conotoxins block channel inactivation. We are examining the influence of Na_Vβ-subunits on the activities of conotoxins by seeing how co-expression of rat Na_Vβ1- through β4-subunits affect the conotoxin-susceptibilites of various rat Na_V1- (or α-) subunits exogenously expressed in *X. laevis* oocytes. We previously reported that co-expression of β-subunits modified the kinetics and voltage-sensitivity of the block of Na_V1.8 by μO-conotoxin MrVIB (Wilson, M. J., et al. 2011. J Pharmacol Exp Ther, 338, 687). More recently, we reported that co-expression of β-subunits altered the kinetics of the block of Na_V1.1, 1.2, 1.6 and 1.7 by μ-conotoxins (principally, μ-TIIIA, μ-PIIIA and μ-SmIIIA); in general, co-expression with either β1 or β3 increased the k_{on} of block whereas co-expression of either β2 or β4 decreased the k_{on} (Zhang, M.-M., et al. 2013 Brit J Pharmacol 168, 1597). We used these results, in part, to help identify the likely Na_V1-isoforms that are functionally expressed on the soma of small and large neurons acutely dissociated from adult rat dorsal root ganglia (DRG) (Zhang, M.-M., et al. 2013 Brit J Pharmacol 169, 102). A major distinguishing feature between β1 or β3 and β2 or β4 is that β2 and β4 are each covalently linked with its associated α-subunit via a disulfide bond, whereas β1and β3 are non-covalently associated with their α-subunit counterparts (Catterall, W. A. 2012 J Physiol 590**,** 2577; Chen, C., et al. 2012 J Biol Chem 287, 39061). Preliminary analyses suggest that the kinetics of the onset of block by μ-SmIIIA of putative Na_V1.7 currents in large DRG neurons are slower than those in small DRG neurons; we are examining this further and suggest that the disparity in kinetics reflects differences in the β-subunits associated with Na_V1.7. Our experience suggests that it should be rewarding to study the effects of β-subunit co-expression on other Na channel ligands; appropriate ones may help in assessing the β-subunit composition of Na channels as well as interactions between α- and β-subunits. Furthermore, knowledge of the pharmacological consequences of β-subunit co-expression may aid in the design of drugs that target Na channels.
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Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.12/D39

Topic: B.04. Ion Channels

Support: DFG RE704/2-1

Title: Use dependent spike shape changes in peripheral cutaneous unmyelinated fibers do not rely on Nav1.8 or Nav1.9

Authors: T. HOFFMANN, K. KISTNER, S. K. SAUER, P. REEH, *C. WEIDNER;
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Abstract: Ionic currents produced during the course of action potential (AP) propagation can be translated into measurable voltage differences, to yield specific waveforms. The shape and size of these waveforms represent biophysical qualities of the cell membrane. It has previously been shown that in rodent DRGs, various voltage dependent currents such as potassium, Ih, TTXs and TTXr sodium currents contribute to the shaping of an AP. Accordingly, both a depolarized membrane potential as well as maintained cell stimulation result in reduction of the AP amplitude and a parallel decrease in conduction velocity (Harty TP et al. 2007, Soleng AF et al. 2003, Blair NT et al. 2003), presumably due to use dependence depletion of sodium influx. Using electrophysiological recordings of single unmyelinated (C) cutaneous fibers and C-fiber compound action potential (CAP) measurements, we evaluated the use dependent waveform changes in mouse peripheral nerves, and the contribution of the TTXr sodium channels Nav1.8 and Nav1.9 to these changes. Nerves from C57Bl6, Nav1.8 KO and Nav1.9 KO mice were electrically stimulated using a single pulse at 2Hz (for CAP experiments) or double pulses at 2Hz (20ms intrastimulus intervals; for single fiber experiments).The induced AP waveforms were divided into several rise/fall sections and for each section, amplitude (i.e., height) as well as duration (i.e., width) were determined.
In all three mice strains, iterative electrical stimulation led to prominent changes in spike waveform. After 120 seconds, the amplitude in both single fibers- and CAP-experiments diminished.
In addition to a reduction in spike amplitudes, waveforms also showed activity dependent increase in spike duration. Waveform changes for all sections were comparable in all three mouse strains.
Upon repetitive electrical stimulation of nerve fibers, slowing of conduction velocity is encountered. This translates into latency shifts and is presumably due to slow inactivation of sodium channels (Carr RW et al. 2008). Accordingly, the electrical protocol used in our study

lead to progressive latency shifts which were comparable in all three mouse strains.
We conclude that in peripheral unmyelinated nerves use dependent waveform changes are neither Nav1.8 nor Nav1.9 dependent.

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Poster

226. **Ion Channels: Na+ Channels**

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Topic: B.04. Ion Channels

Support: Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs

Erythromelalgia Association

"Profileringsfonds" University Hospital Maastricht

Title: Small-fiber neuropathy Nav1.8 mutation shifts activation to hyperpolarized potentials and increases excitability of DRG neurons

Authors: *J. HUANG[1,2], Y. YANG[1,2], P. ZHAO[1,2], M. GERRITS[3], J. HOEIJMAKERS[4], K. BEKELAAR[4], I. MERKIES[4,5], C. FABER[4], S. DIB-HAJJ[1,2], S. WAXMAN[1,2];
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Abstract: Idiopathic small-fiber neuropathy (I-SFN), clinically characterized by burning pain in distal extremities and autonomic dysfunction, is a disorder of small caliber nerve fibers, unknown etiology with limited treatment options. Recently, functional variants of the peripheral voltage-gated sodium channel $Na_v1.7$, encoded by *SCN9A*, were identified in about one-third of I-SFN patients. These variants were shown to render dorsal root ganglion (DRG) neurons hyperexcitable. Like $Na_v1.7$, sodium channel $Na_v1.8$, encoded by *SCN10A*, is preferentially expressed in small-diameter DRG neurons, and has been shown to be responsible for most of the transmembrane current underlying the upstroke of action potentials in these neurons. We previously demonstrated functional variants of $Na_v1.8$ that either enhance ramp current or shift

activation in a hyperpolarizing direction, and render DRG neurons hyperexcitable in several I-SFN patients with no mutations of *SCN9A*. We have now evaluated additional I-SFN patients with no mutations in *SCN9A*, by sequencing *SCN10A*. We have identified a novel I-SFN related $Na_v1.8$ mutant and tested it functionally using whole-cell voltage-clamp and current-clamp recordings in small DRG neurons. Compared to the wild-type, mutant channels show a significant hyperpolarizing shift in $V_{1/2}$ of activation and in the voltage for the peak ramp current. The mutation does not affect the $V_{1/2}$ or slope factor of steady-state fast inactivation, but it decreases the percentage of non-inactivating channels, probably associated with a smaller persistent current. Current-clamp studies reveal that the mutation negatively shifts the voltage threshold of action potential and depolarizes the resting membrane potential, and reduces the current threshold and increases the firing frequency of evoked action potentials. These observations suggest that the effects on activation and ramp current are dominant over the reduced persistent current amplitude, and these pro-excitatory gating changes confer hyperexcitability on peripheral sensory neurons, which may contribute to pain in this individual with I-SFN.

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Poster

226. **Ion Channels: Na+ Channels**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.14/D41

Topic: B.04. Ion Channels

Support: Research agreement with UCB Pharmaceuticals.

Title: Lacosamide modulation of the voltage-gated sodium channel $Na_v1.1$

Authors: D. H. FELDMAN[1], S. S. SHAHANGIAN[1], S. HIROSE[2], M. A. ROGAWSKI[1], *C. LOSSIN[1];
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Abstract: Lacosamide is a new generation anticonvulsant drug targeting voltage-gated sodium (Na_v) channels. Its mode of action is unique in that it primarily blocks Nav channels under high-frequency firing conditions while maintaining the normal physiological activity of the channels. This is possible through selective enhancement of Na_v channel slow inactivation, a state entered

mainly when the channels are stimulated for prolonged periods of time. Biophysical characterizations of lacosamide blockade are now available for several Na_v channel isoforms. However, the effects on one particularly important player in epilepsy, $Na_v1.1$, remain unknown. Variations in the $Na_v1.1$ gene, *SCN1A*, lead to several types of febrile seizures, including those where status epilepticus is a hallmark component of the presentation. To get a more complete understanding of lacosamide's mechanism of action, we subjected heterologously expressed $Na_v1.1$ to various lacosamide concentrations. Consistent with previous reports, we found a hyperpolarizing shift in the voltage-dependence of slow inactivation, reducing $Na_v1.1$ activity at less depolarized potentials compared to when the drug is absent. We furthermore observed a shift in the onset of slow inactivation, which makes $Na_v1.1$ enter slow inactivation more readily when lacosamide is present. Use dependence of the channel was clearly enhanced, particularly at higher frequencies.

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Poster

226. **Ion Channels: Na+ Channels**

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Topic: B.04. Ion Channels

Support: Mision Ciencia 200700642 (Venezuela)

Title: Tctx2 and Tctx3 : Tityus caripitensis toxins with effect over Nav1.4 channels

Authors: *Y. D. HERNANDEZ ESTEVEZ[1,2], C. CASTILLO[1];
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[2]Postgrado en Ciencias Biologicas, Univ. Simon Bolivar, Caracas, Venezuela, Bolivarian Republic of

Abstract: Scorpion venoms are a rich mixture of compounds and had been broadly studied as a source of peptides known as toxins. These toxins have effect over sodium, potassium, calcium

and chloride voltage gated channels. Toxins that affect sodium channels have 60-76 amino acids and are stabilized by four disulfide bridges. They have been traditionally classified in two groups: alpha-toxins and beta-toxins, both are structural and functionally different. In one hand, alpha-toxins bind to site 3 of sodium channel and modify the inactivation kinetic of sodium channels maintaining the channels open. On the other hand, beta-toxins bind to site 4 and modified activation kinetic by shifting the voltage dependence of activation to more negative potentials, induce repetitive and spurious firing of action potentials and reduce the peak conductance. One of the species related with scorpionism accidents in Northeastern Venezuela is *Tityus caripitensis*. The venom of *Tityus caripitensis* was fractionated using SE-HPLC on a Protein Pak 60 column. The most abundant fraction was further separated by RP-HPLC on an analytical C18 column and the fractions named Tctx2 and Tctx3 were upon a refined RP-HPLC yielded pure peptides. We determined their molecular weight by MALDI-TOF (mode reflector) of 7702 and 7683 Da respectively. Finally, we use whole cell clamp to evaluate the effect of both toxins over Nav1.4 channels expressed in HEK293 cells. We found that in the presence of 130nM of Tctx2 the conductance of the Nav1.4 channel decreased 45% while in presence of 130nM of Tctx3 the conductance is decreased by 69%. The midpoint potential of the steady state activation curve were shifted to the left by 10mV ± 1.4 in presence of Tctx2 (n=3) and by 8mV ± 1.2 in presence of Tctx3 (n=3). Tctx2 and Tctx3 are the first beta-toxins isolated from *Tityus caripitensis* venom.

Disclosures: Y.D. Hernandez Estevez: None. **C. Castillo:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.16/E1

Topic: B.04. Ion Channels

Support: NIH Grant MH095995

Title: Functional regulation of the Nav1.2 channel by glycogen synthase kinase 3

Authors: *T. F. JAMES[1], J. LUISI[2], M. N. NENOV[2], N. PANOVA[2], F. LAEZZA[2];
[2]Pharmacol. and Toxicology, [1]Univ. of Texas Med. Br., Galveston, TX

Abstract: Dysregulation of glycogen synthase kinase 3β (GSK3β) has been associated with several disorders, including Alzheimer's disease, addiction, and mood disorders and may be fundamentally linked to disorders of excitability in brain circuits. We hypothesized that a critical link between GSK3β and brain disorders may arise from regulation of voltage-gated sodium

(Na_v) channels that are critical molecular determinants of neuronal excitability. To test this hypothesis, we used patch clamp electrophysiology to record sodium currents from $Na_v1.2$ channels stably expressed in HEK293 cells. We found that pharmacological inhibition of GSK3 with GSK3 inhibitor XIII potentiated the peak current density of $Na_v1.2$ from -56.91 ± 10.60 pA/pF (0.05% DMSO; n=10) to -115.44 ± 17.51 pA/pF (10 µM GSK3 inhibitor XIII; n=14, p=0.01) and shifted V1/2 of the voltage dependence of steady-state inactivation from -63.79 ± 2.05 mV (n=9) to -54.14 ± 0.67 mV (n=15, p=0.001). To explore the notion that GSK3 may modulate the expression level of these channels, we used confocal microscopy to analyze the $Na_v1.2$ protein expression within the cell. Our results indicate that exposure to GSK3 inhibitor XIII induced a significant increase in $Na_v1.2$ channel expression when compared to findings following DMSO treatment (n>80 cells, p<0.0001). Additional confocal studies were performed to analyze the cell surface expression of a CD4 chimeric construct expressing the C-terminal tail of the $Na_v1.2$ channel (CD4-$Na_v1.2$-C-tail) in DMSO versus GSK3 inhibitor XIII. Phenotypic changes in fluorescence intensity distributions relative to surface labeling between the two groups indicate that inhibition of GSK3 might affect membrane trafficking of the $Na_v1.2$ channel through its C-terminal tail. Taken together, these findings indicate that GSK3 modulates pathways affecting Na_v channel expression and function, broadening the repertoire of possible targets for future therapeutic interventions against its associated disorders.

Disclosures: T.F. James: None. **J. Luisi:** None. **M.N. Nenov:** None. **N. Panova:** None. **F. Laezza:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.17/E2

Topic: B.04. Ion Channels

Support: NIH Grant RO1 MH095995 (FL)

Title: Regulation of the Nav1.6 channel by glycogen synthase kinase 3 (GSK3)

Authors: *M. N. NENOV, N. I. PANOVA, F. LAEZZA;
Dept. of Pharmacol. and Toxicology, The Univ. of Texas Med. Br., Galveston, TX

Abstract: GSK3 is an evolutionarily conserved ubiquitous kinase known to control normal brain function through an intricate network of intracellular signaling pathways. Evidence indicates that dysfunctional activity of this kinase correlates with psychiatric disorders, addictive behaviors, and neurodegenerative diseases. However, the detailed mechanisms of GSK3 action on neuronal

functioning are still poorly understood. Exciting new evidence indicates that neuronal ion channels are new promising targets of this enzyme. In support of this, we recently demonstrated that inhibition of GSK3 reduces the assembly of the voltage-gated sodium channel Nav1.6 with its regulatory protein, the fibroblast growth factor 14 (FGF14), modifying FGF14-dependent regulation of Nav1.6 currents (Shavkunov et al., J Biol Chem., 2013 in press). Building on these results, we posited that GSK3 might also exert a direct action on the Nav1.6 channel in the absence of any regulatory proteins. To test this hypothesis we applied whole-cell patch-clamp to record sodium currents from HEK293 cells stably expressing human Nav1.6 channels. Our results indicate that pharmacological inhibition of GSK3 (GSK3 inhibitor XIII, 30 uM) induces a significant reduction ($p<0.05$) of Nav1.6 peak current density (-29.3±3.9 pA/pF, n=8) compared to control (-47.2± 6.5 pA/pF, n=8). Furthermore, we show that inhibition of GSK3 produces a significant shift ($P<0,005$) in the steady-state inactivation of the Nav1.6 channel (-68.9±1.7, n=6) compared to control (-58.8±1.6, n=7,) without apparent changes in the voltage-dependence of activation. Taken together, these findings provide evidence for a complex role of GSK3 on neuronal Nav channels which is expected to greatly impact neuronal excitability in brain circuits.

Disclosures: M.N. Nenov: None. **N.I. Panova:** None. **F. Laezza:** Other; Center for Addiction Research, Center for Biomedical Engineering, Mitchell Center for Neurodegenerative Diseases.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.18/E3

Topic: B.04. Ion Channels

Support: NIH Grant NS064274

NIH Grant NS072040

Title: Lacosamide inhibition of Nav1.7 voltage-gated sodium channels: Binding to fast-inactivated versus slow-inactivated states

Authors: *S. JO, B. BEAN;
Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Lacosamide (Vimpat) is an anti-epileptic agent that appears to be targeted to voltage-dependent sodium channels. Previous experiments suggested that lacosamide is unusual in having selective binding to the slow-inactivated state of sodium channels, in contrast to other anti-epileptic drugs that bind tightly to fast-inactivated states of the channel. We examined the

state-dependent effects of lacosamide on human Nav1.7 sodium channels, studied in a stable cell line. In agreement with previous results, inhibition by lacosamide was highly voltage-dependent. 100 μM lacosamide inhibited sodium current elicited from a holding potential of -120 mV by 3 ± 1%, current elicited from -80 mV by 43 ± 4%, and current elicited from -70 mV by 61 ± 1%. However, in contrast to previous results, we found that lacosamide induced a reversible shift in the voltage-dependence of fast-inactivation studied with 100 ms prepulses, suggesting that the compound can bind tightly to fast-inactivated as well as slow-inactivated states. To explore this issue further, we used a protocol examining the rate of entry of channels into slowly-recovering states at either -40 mV, where fast-inactivation is complete but slow-inactivation is not, or 0 mV, where slow-inactivation is maximal. At -40 mV, in control 24 ± 4% of channels entered slowly-recovering states after 16 seconds, and this was increased substantially to 49 ± 5% of channels in the presence of 100 μM lacosamide. At 0 mV, in control 48 ± 4% of channels entered slowly-recovering states after 16 seconds, and this was increased relatively little, to 52 ± 7% of channels, in the presence of 100 μM lacosamide. The results of these and other experiments seem more compatible with lacosamide binding slowly to fast-inactivated states (and possibly also to slow-inactivated states) than with exclusive binding to slow-inactivated states. Comparisons to carbamazepine (fast binding to fast-inactivated states), phenytoin (slow binding to fast-inactivated states) and brilliant blue G (binding to both fast-and slow-inactivated states) will be presented.

Disclosures: S. Jo: None. **B. Bean:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.19/E4

Topic: B.04. Ion Channels

Support: Medical Research Council

Royal Society University Research Fellowship

Title: Conserved functional impact of alternative splicing in domain 1 of neuronal sodium channels

Authors: A. LIAVAS, *S. SCHORGE;
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Abstract: A conserved splicing event in sodium channel genes gives rise to two alternative splice variants, the so-called neonatal (N) and adult (A) forms. This splicing may have clinical impact, as a polymorphism in the SCN1A gene that modifies the consensus sequence of the neonatal exon (5N) has been linked to altered seizure predisposition or to altered anti-epileptic drug (AED) dosage in epileptic patients; however these associations have not been replicated in all populations. The functional impact of the splicing has not been systematically compared in different channel backgrounds. We asked whether the functional effects seen in NaV1.1 channels, which are thought to dominate in interneurons of the CNS, can be replicated by splice variants of NaV1.7 channels, which are important in peripheral pain-sensing neurons.
Previously we reported that heterologously expressed NaV1.1-5N channels in HEK cells recover more quickly from fast inactivation than NaV1.1-5A channels. Here, we use similar whole cell patch clamp recordings to characterize the conditions that maximise this difference. Recovery rate is influenced by temperature, voltage, duration of the gap between the first (P1) and recovery (P2) depolarizing steps, and by the length of the first depolarising step (P1). The difference between splice variants seems to be most robust at physiological temperature, with short P1 intervals, and short gap durations. Furthermore, the difference is augmented by consecutive rapid pulse protocols that are consistent with the sort of fast neuronal activity that might occur during epileptic events. In contrast, the difference between splice variants is masked by prolonged depolarizing P1 steps or by recovery intervals longer than 200ms. To test whether the functional impact of the conserved splicing event is also conserved, we expressed the closely-related NaV1.7-5A and NaV1.7-5N splice variants. We found that although NaV1.7 shows a more depolarised voltage-dependence of inactivation than NaV1.1, the splice variants of NaV1.7 preserved the difference in recovery from inactivation seen for variants of NaV1.1, with the neonatal variant recovering more quickly in these channels after a range of depolarizing pre-pulses.
Our data indicate that, in NaV channels, a molecular conservation of splicing between channel subtypes may lead to a conserved functional change across channel subtypes. Moreover, splicing may have its biggest impact on inactivation in conditions that could mimic rapid firing patterns, such as fast activity during a seizure or in interneurons.

Disclosures: A. Liavas: None. **S. Schorge:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.20/E5

Topic: B.04. Ion Channels

Support: CNPq

CAPES

FUNCAP

Title: Inhibitory excitability effect by terpinen-4-ol is related to both blocked sodium and potassium voltage-gated channels in small dissociated dorsal root ganglia neurons

Authors: *T. SANTOS-NASCIMENTO, K. MORAIS VERAS, L. MOREIRA-JUNIOR, J. LEAL-CARDOSO;
State Univ. of Ceara, Fortaleza, Brazil

Abstract: Terpinen-4-ol (4TERP) is a monoterpenoid with extensive commercial use, with several pharmacological activities and with potential therapeutic interest, including depressant effects on the nervous system. The present study investigated whether 4TERP alters neuronal excitability through effects on both Na+ (INa) and K+ (IK) voltage-gated currents in neurossoma of dorsal root ganglia (DRG). We used preparations of intact and dissociated neurons of rat DRG for intracellular and patch-clamp recordings. At 6 mM, 4TERP blocked the generation of action potentials (AP) and depolarized ≈ 6 mV (n=7) of resting potential (control -59.09 ± 2.27 mV; 4TERP -53.19 ± 2.28 mV) of neurons of intact DRG. At 1 mM, 4TERP decreased rising (dV/dt)max and increased AP duration. In dissociated neurons, 4TERP inhibited ≈ 50% total INa (IC50 was 0.8 ± 0.3 mM) and ≈ 20% TTX resistant INa (IC50 was 300 ± 300 μM). 4TERP also inhibited ≈ 60% of two 4-aminopiridin sensitive K+ current: the transient (Ia, IC50 was 3.2 ± 0.0 mM) and steady (Id, IC50 was 700 ± 100 μM). 4TERP also inhibited ≈ 85% of steady TEA sensitive K+ current (Ik, IC50 was 1.6 ± 0.7 mM). 4TERP shift to the right the activation curve and did not alter inactivation curve of the all currents tested. In conclusion, we demonstrated that 4TERP blocks neuronal excitation and its effect on Na+ and K+ currents is coherent with its activity on nervous excitability. This effect of 4TERP on currents is relevant to the explanation of several other effects of 4TERP reported on the literature.

Disclosures: T. Santos-Nascimento: None. **K. Morais Veras:** None. **L. Moreira-Junior:** None. **J. Leal-Cardoso:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.21/E6

Topic: B.04. Ion Channels

Support: NIH/NIGMS R01-GM095430

Title: A-type fibroblast growth factor homologous factors function as open channel blockers to modulate voltage-gated sodium channel availability

Authors: *Y. LIU, M. GOLDFARB;
Hunter College, City Univ. of New York, New York, NY

Abstract: Action potential generation is controlled by the opening, inactivation, and recovery of voltage-gated sodium channels (Na_V). A-type fibroblast growth factor homologous factors (A-type FHFs), which are cytosolic Na_V binding proteins, mediate rapid-onset, long-term inactivation (LTI) of Na_V by contributing an independent inactivation particle that competes with the Na_V intrinsic fast inactivation mechanism (Dover et al., J. Physiol. 588:3695; 2010). To further explore the structural mechanism of A-type FHF induced LTI of Na_V, we have performed cotransfection of wild-type and mutant FHF2A expression plasmids together with wild-type and mutagenized TTX-resistant Nav1.6 and Nav1.5 expression plasmids into Neuro2A cells, and have analyzed TTX-resistant sodium currents through various whole cell voltage clamp protocols. Our results demonstrate that LTI induced by FHF2A only occurs when the channels reach the open state. FHF2A can compete for binding to channels with another open channel blocking protein, Na_V[[Unsupported Character - Symbol Font ]]4, which is responsible for resurgent sodium current due to its rapid dissociation from channel upon repolarization. Both FHF2A and Na_V[[Unsupported Character - Symbol Font ]]4 blocking particles rely upon hydrophobic and basic residues, suggesting they may share a common binding surface within Na_V's cytoplasmic cavern exposed in the open state. However, FHF2A dissociates much more slowly from channels than does Na_V[[Unsupported Character - Symbol Font ]]4, accounting for their functional dissimilarity. Ongoing channel mutagenesis seeks to reveal channel binding sites for each of these blocking particles. In summary, our studies show that A-type FHFs serve as open sodium channel blockers, in competition with both the Na_V intrinsic fast inactivation mechanism and the accessory subunit Na_V[[Unsupported Character - Symbol Font ]]4, to drive channels into a long-term refractory state.

Disclosures: Y. Liu: None. **M. Goldfarb:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.22/E7

Topic: B.04. Ion Channels

Support: NIH Grant NS039579

Title: Gating properties of somatic voltage-gated sodium currents in layer 4 primary cells and interneurons of the somatosensory cortex

Authors: *Y. MA[1], M. GUTNICK[2], D. PRINCE[1], J. HUGUENARD[1];
[1]Stanford Univ., Stanford, CA; [2]The Hebrew Univ. of Jerusalem, Rehovot, Israel

Abstract: In somatosensory cortex, neurons of Layer 4 are the primary recipients of thalamocortical input. Because somatic Na^+ channels play a pivotal role in regulating membrane and cellular excitability, study of their kinetic properties and functions is important for understanding thalamocortical information processing. Using the nucleated patch clamp recording technique in acute cortical slices, we have quantified the kinetics of somatic Na^+ channels in identified regular-spiking excitatory (RS) cells, fast-spiking (FS) inhibitory cells, and somatostatin-containing (SOM) inhibitory cells. We now describe our data in terms of the classical Hodgkin-Huxley formalism. Because voltage-dependence of transfer rates for channel opening (α) and closing (β) are the two fundamental factors determining the channel behavior as a function of voltage and time, we optimized the equations to describe α and β as a function of voltage for each of the cell types, and constructed steady state activation (m_∞) and inactivation (h_∞) curves. curves. Activation kinetics were similar for all three cell types, while the h_∞ curve was slightly right-shifted in FS interneurons, and markedly right shifted in SOM cells. Thus, the SOM cells had a significantly greater window current ($m_\infty^3 * h_\infty$). The higher steady state Na^+ channel open probability near resting potential suggests that the excitability of layer 4 SOM cells at subthreshold voltages is tightly regulated by voltage-gated Na^+ currents.

Disclosures: Y. Ma: None. **M. Gutnick:** None. **J. Huguenard:** None. **D. Prince:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.23/E8

Topic: B.04. Ion Channels

Support: NIH R01NS047506

R01NS066027

S21MD000101

U54 RR026137

T32HL103104

ALZ IIRG-10-173350

Title: Acid-sensing ion channels in NS20Y cells - potential role in neuronal differentiation

Authors: Z. O'BRYANT, T. LENG, K. INOUE, Z. ZENG, *Z.-G. XIONG;
Neurosci. Inst., Morehouse Sch. of Med., Atlanta, GA

Abstract: Cultured neuronal cell lines can express many properties of mature neurons if properly differentiated. Although the precise mechanisms underlying neuronal differentiation are not fully understood, expression and activation of ion channels, particularly Ca^{2+}-permeable channels, have been suggested to play an important role in the process. NS20Y, a neuronal cell line derived from the mouse neuroblastoma, has been used as an *in vitro* model for the study of neuronal differentiation. However, there has been a lack of extensive characterization of the expression and properties of various ion channels in these cells. Using patch-clamp recording and RT-PCR technique, we explored the existence of acid-sensing ion channels (ASICs), a novel family of proton-gated cation channels, in NS20Y cells. Fast drops of extracellular pH activated transient inward currents with a $pH_{0.5}$ at ~6.0. Currents in NS20Y cells were blocked by amiloride, a non-selective ASIC blocker, and PcTX1, a specific inhibitor for homomeric ASIC1a and heteromeric ASIC1a/2b channels. RT-PCR demonstrated the existence of ASIC1a transcript in these cells. Other pharmacological characteristic such as potentiation by zinc chelation also supports the presence of functional homomeric ASIC1a channels. Potential function of ASIC1a channels in the growth and differentiation of NS20Y cells is under investigation.

Disclosures: Z. O'Bryant: None. **Z. Xiong:** None. **T. Leng:** None. **K. Inoue:** None. **Z. Zeng:** None.

Poster

226. **Ion Channels: Na+ Channels**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 226.24/E9

Topic: B.04. Ion Channels

Title: Bace 1 regulates the voltage gated sodium channel Nav 1.7

Authors: X. REZAI, V. CURTO-REYES, *I. DECOSTERD;
CHUV and Lausanne Univ., Lausanne, Switzerland

Abstract: Voltage gated sodium channels (Nav) are responsible for the generation and transmission of action potential. In dorsal root ganglion neurons, the alpha subunit Nav 1.7 is crucial for the transmission of nociceptive information. Following nerve injury, Nav 1.7 expression is altered, a process that has been associated with the development of neuropathic pain. We demonstrated previously an important regulatory role the auxiliary beta subunits in the dysregulation of Nav channel expression during neuropathic pain. In addition, it has been shown that beta subunits can be cleaved by BACE1, a beta-secretase best known for cleaving APP in the context of Alzheimer's disease, thereby regulating the amount of channel expressed and stabilized at the plasma membrane but also directly modulating Nav channel properties.
Here, we co-expressed human BACE1, Nav 1.7 and different beta subunits in HEK cells. BACE1 mutants with and without catalytic site have also been used. Western blots and whole cell electrophysiological recording of sodium currents were performed to study modification of Nav 1.7 expression and current carrying properties induced by the presence of BACE1, alone or in presence of beta subunits.
Results show that Nav 1.7 current carrying properties are modified in the presence of BACE1, a process that seems to be independent from the ability of the enzyme to cleave the beta subunits. Nav 1.7 total protein level was not modified in the presence of BACE1 alone or co-expressed with the Beta2 subunit. We are carrying now cell biotinylation in order to quantify Nav1.7 expression at the plasma membrane.
Overall our results suggest that Nav 1.7 properties are regulated by BACE1. Our future experiments will determine if this regulation is relevant in the context of neuropathic pain. This highlights a potential therapeutical interest in parallel to the tremendous effort spent to develop specific sodium channel blockers.

Disclosures: X. Rezai: None. **I. Decosterd:** None. **V. Curto-Reyes:** None.

Poster

227. **Monoamine Transporters: Function and Modulation**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 227.01/E10

Topic: B.05. Transporters

Support: NSF IOS 0922085 (MO)

NSF IOS 0921969 (CAL)

NSF IOS 0921874 (KJR)

Title: Resolving the transport kinetics of the corticosterone-sensitive organic cation transporter 3 (OCT3/Slc22a3) in the male rat brain

Authors: *J. S. TALBOOM[1,2], J. MOLINARO[1], C. A. LOWRY[3], K. J. RENNER[4], M. ORCHINIK1[1];
[1]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [2]Arizona Alzheimer's Consortium, Phoenix, AZ; [3]Dept. of Integrative Physiol., Univ. of Colorado Boulder, Boulder, CO; [4]Dept. of Biol., Univ. of South Dakota, Vermillion, SD

Abstract: The organic cation transporter 3 (OCT3/Slc22a3) is a low affinity and high capacity polyspecific monoamine transporter found in the human and rodent brain. OCT3 is thought to clear monoamines (e.g., serotonin, norepinephrine, & dopamine) from extracellular fluid in the brain, thereby helping to terminate their signal after release. OCT3 may complement other specific (e.g., SERT, DAT, & NET) or polyspecific (e.g., PMAT, OCT1, & OCT2) transporters in regions of the brain that are innervated by monoaminergic terminals. OCT3 is a unique transporter in that it is the only known monoamine transporter inhibited by physiological levels of corticosterone (CORT) in the rat. Among other stress-related neural processes, CORT-mediated inhibition of OCT3 may modulate the hypothalamic-pituitary-adrenocortical (HPA) axis via alterations in monoaminergic neurotransmission. We hypothesize that OCT3 functions in brain regions regulating the HPA axis to increase extracellular monoamine concentrations during the acute stress response. Previous studies determining the transport kinetics of OCT3 have been contradictory. Data collected from a cell line expressing OCT3, as compared to tissue minces of the rat hypothalamus, suggested a several fold difference in the potency of CORT to inhibit OCT3-mediated transport. We set out to resolve these conflicting reports and confirm that physiological levels of CORT can inhibit OCT3-mediated transport in intact brain sections. To accomplish this, we quantified the uptake of 4-(4-dimethylaminostyryl)-N-methylpyridinium (ASP^+), a fluorescent organic cation, in live ciliated ependymal cells lining the ventral third cerebral ventricle (V3V). Three hundred μm live brain sections from male Sprague Dawley® rats (~250 g), containing the V3V (adjacent to the dorsomedial hypothalamus), were collected in artificial cerebrospinal fluid equilibrated with O_2 and CO_2. Sections were placed in a perfusion chamber under the laser scan head of an Olympus confocal microscope equipped with a water immersion objective. ASP^+ was excited by a 488 nm Ar laser and optical sections were acquired; our dependent measure was the relative fluorescent intensity of the V3V ependymal cells across time. Separate brain sections from multiple animals were treated with different transporter inhibitor solutions in order to determine OCT3-mediated ASP^+ uptake. Iterative curve-fitting techniques were used to determine the transport kinetics of OCT3 as well as the IC_{50}s of the inhibitors. The results indicated a CORT-induced inhibition of OCT3-mediated ASP^+ uptake. Studies of this nature may lead to a better understanding of the function of OCT3 in the brain.

Disclosures: J.S. Talboom: None. **J. Molinaro:** None. **C.A. Lowry:** None. **K.J. Renner:** None. **M. Orchinik1:** None.

Poster

227. **Monoamine Transporters: Function and Modulation**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 227.02/E11

Topic: B.05. Transporters

Support: NIEHS P01 ES016731

NIH T32 ES012870

NIH T32 ND015040

Title: The vesicular monoamine transporter 2 (VMAT2) as a mediator of vesicular function, neurotoxicity and behavior

Authors: *K. M. LOHR[1], A. I. BERNSTEIN[1], K. A. STOUT[1], A. R. LUCE[1], T. S. GUILLOT[1], M. WANG[1], Y. LI[1], A. SALAHPOUR[2], G. W. MILLER[1];
[1]Envrn. Hlth., Emory Univ., Atlanta, GA; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: The vesicular monoamine transporter 2 (VMAT2) is responsible for packaging monoamines into vesicles for rapid release at the synapse. In addition to its role in neurotransmission, VMAT2 is also responsible for sequestering toxicants away from their sites of action in the cell. Our lab has previously characterized mice with reduced vesicular capacity, which results in progressive degeneration of monoaminergic neurons and both motor and non-motor deficits (Caudle et al., JNS 27:8138, 2007; Taylor et al., JNS 29:8103, 2009). Despite ample evidence suggesting that decreased VMAT2 levels results in neuronal dysfunction, it was not known if an increase in VMAT2 level would result in increased function in an animal model. We created a BAC transgenic VMAT2 overexpressing (VMAT2HI) mouse line to determine the effects of increased vesicular monoamine filling on associated neurochemical, behavioral, and toxicological outcomes. Our findings using these VMAT2HI mice suggest that the increased vesicular storage mediated by increased VMAT2 levels can alter both the neurochemical and behavioral output of the monoaminergic system. In addition to increased vesicular uptake, VMAT2HI mice also show a 75% increase in locomotor activity during the active period and improved outcomes on measures of anxiety and depressive-like behaviors, including a 42% reduction in marble burying and a 22% decrease in immobility time on the forced swim test. Further, since VMAT2 serves as a neuroprotective mechanism in monoaminergic systems, our VMAT2 overexpressing mice also serve as a valuable tool to investigate resistance to both endogenous and exogenous toxicant exposure. Preliminary evidence suggests that VMAT2HI mice have reduced indices of cytosolic dopamine turnover in the striatum and cortex (as

indicated by the metabolite/transmitter ratio of DOPAC to DA by HPLC). Supported by NIEHS P01 ES016731, T32 ES012870, and T32 ND015040.

Disclosures: K.M. Lohr: None. **A.I. Bernstein:** None. **K.A. Stout:** None. **A.R. Luce:** None. **T.S. Guillot:** None. **M. Wang:** None. **Y. Li:** None. **A. Salahpour:** None. **G.W. Miller:** None.

Poster

227. **Monoamine Transporters: Function and Modulation**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 227.03/E12

Topic: B.05. Transporters

Support: NIEHSP01ES016731

T32ES012870

T32ND015040

Title: Genetic manipulation of vesicular transport machinery mediates dopamine neurochemistry and release dynamics

Authors: *A. LUCE[1], K. M. LOHR[1], K. A. STOUT[1], A. I. BERNSTEIN[1], T. S. GUILLOT, III[1], T. S. GUILLOT, III[1], M. WANG[1], Y. LI[1], A. SALAHPOUR[3], G. W. MILLER[1,2]; [1]Envrn. Hlth., [2]Ctr. for Neurodegenerative Dis., Emory Univ., Atlanta, GA; [3]Pharmacol. and Toxicology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Proper vesicular function is critical for dopamine neuron health and integrity. The vesicular monoamine transporter 2 (VMAT2) is responsible for packaging monoamines such as dopamine into synaptic vesicles. This readies the synapse for rapid dopaminergic neurotransmission. Impairing function of the vesicle by reducing VMAT2 levels leads to an excess of cytosolic dopamine, which is toxic to the cell. This leads to dopaminergic degeneration and motor and nonmotor deficits in mouse models of VMAT2 underexpression, as characterized previously by our lab. These characteristics are similar to human Parkinson's disease, of which dopamine neurodegeneration and motor and nonmotor deficits are key components. Although there is ample evidence that impaired vesicular function resulting from VMAT2 deficiency leads to dopamine mishandling and cell death, the converse_enhanced vesicular function_had been previously unexplored. The current investigation explores the protective potential of overexpressing VMAT2. We utilize a bacterial artificial chromosome (BAC) transgenic mouse

model to overexpress VMAT2 (VMAT2HIs) and various outcome measures including radioactive vesicular uptake, ex vivo fast scan cyclic voltammetry and analysis of vesicular morphology by electron microscopy to determine the effect of VMAT2 overexpression on dopamine handling characteristics and neurochemistry. Results from these experiments indicate that enhanced vesicular function resulting from VMAT2 overexpression increases vesicular capacity for dopamine. First, we observed a 12% increase in average vesicular diameter in VMAT2HI animals, which corresponds to a 50% increase in vesicular volume. This larger vesicular capacity for dopamine is thought to underlie observed neurochemical changes. We found a two-fold increase in vesicular uptake of dopamine that is reversible by reserpine, as well as an increase in stimulated dopamine release by voltammetry. We hypothesize that this enhanced vesicular function confers resistance to endogenous and exogenous toxic insults.
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Poster

227. **Monoamine Transporters: Function and Modulation**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 227.04/E13

Topic: B.05. Transporters

Support: NIH K08-GM094394

NIH TR01-GM104948

Title: Dextroamphetamine induces reanimation from sevoflurane general anesthesia by enhancing dopaminergic neurotransmission

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Abstract: BACKGROUND
Previous studies show that methylphenidate induces reanimation (i.e. active emergence) from general anesthesia in rodents. The objective of this study was to probe the relative contributions of dopaminergic vs. noradrenergic neurotransmission to reanimation from general anesthesia.
METHODS
Male Sprague-Dawley rats were anesthetized with sevoflurane, and an IV catheter was placed in

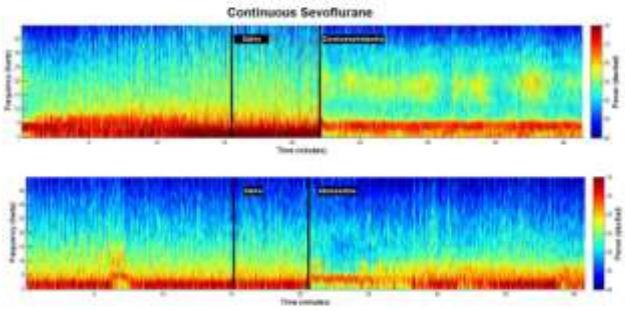
a lateral tail vein. The animals were placed supine in an anesthetizing chamber with a port for IV access. The dose of sevoflurane sufficient to maintain loss of righting was established and fixed prior to the administration of dextroamphetamine (n=6) or the selective norepinephrine transporter inhibitor atomoxetine (n=6). An additional group of rats with extradural EEG electrodes (n=3) received dextroamphetamine or atomoxetine during continuous sevoflurane general anesthesia, and spectrograms were computed from the EEG recordings.

RESULTS

During continuous sevoflurane general anesthesia, dextroamphetamine restored conscious behaviors including the righting reflex in a dose-dependent manner, whereas atomoxetine did not induce an arousal response. Pretreatment with the D1 dopamine receptor antagonist SCH-23390 greatly attenuated dextroamphetamine-induced arousal, and inhibited the return of righting in 5/6 rats. Spectrograms computed from EEG recordings revealed a prompt shift in peak power from delta (<4 Hz) to theta (4-8 Hz) after intravenous administration of dextroamphetamine or atomoxetine (Fig. 1).

CONCLUSIONS

Dextroamphetamine restores conscious behaviors including the righting reflex during continuous sevoflurane anesthesia. The primary mechanism of action is likely via activation of dopaminergic (rather than noradrenergic) arousal circuits. However, similar EEG changes were induced by dextroamphetamine and atomoxetine, suggesting that the shift in EEG power from delta to theta is mainly driven by noradrenergic stimulation. These findings suggest that dextroamphetamine may be clinically useful to restore consciousness in surgical patients.



Disclosures: J.D. Kenny: None. **E.N. Brown:** None. **K. Solt:** None.

Poster

227. **Monoamine Transporters: Function and Modulation**

Location: Halls B-H

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Program#/Poster#: 227.05/E14

Topic: B.05. Transporters

Support: Grant-in-aid for Scientific Research from the Ministry of Education, Culture, Science, Sports and Technology

Smoking Research Foundation

Title: Regulation of noradrenaline transporter gene expression by nicotine

Authors: *N. SOGAWA, C. SOGAWA, K. OHYAMA, X.-F. WEN, D. YI, S. KITAYAMA; Dent. Pharmacol., Okayama Univ. Grad. Sch. Med. Dent. Pharmaceut. Sci., Okayama, Japan

Abstract: Nicotine, a major alkaloid constituent in tobacco, is a potent sympathomimetic stimulant, and is believed to be responsible for the pathogenesis of cardiovascular diseases associated with cigarette smoking. Nicotine increases sympathetic nerve and adrenomedullary activity, which underlies the pathogenesis of cardiovascular diseases associated with cigarette smoking. One possible target of nicotine is the reuptake system for catecholamines, such as noradrenaline transporter (NET). Uptake through the plasma membrane NET is the primary mechanism for regulating extracellular noradrenaline concentrations, and is, thus, a key player in controlling sympathetic nerve activity. NET belongs to the Na^+ and Cl^--dependent neurotransmitter transporter gene family, and is a target of various drugs acting on the central nervous system, including psychostimulants. However, there is little evidence for nicotinic modulation of the uptake system including NET. We investigated the effects of nicotine on NET expression in PC12 cells derived from rat pheochromocytoma, human neuroblastoma SK-N-SH cells, and rat tissues. Nicotine had no influence on the transcriptional activity of human NET gene constructs transiently transfected in SK-N-SH and PC12 cells. However, nicotine decreased NET mRNA levels within several hours in SK-N-SH cells. In contrast, nicotine up-regulated the mRNA and protein of NET in PC12 cells. A single administration of nicotine increased NET mRNA levels in the adrenal medulla and brain stem of the rat, whereas chronic nicotine treatment for 2 weeks decreased NET mRNA levels. These results suggest that nicotine up- and down-regulates the expression of NET through a posttranscriptional mechanism. The decrease in NET expression induced by nicotine may be of particular clinical relevance to the pharmacological action of nicotine.
This study was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Education, Culture, Science, Sports and Technology (KO) and a grant from the Smoking Research Foundation (CS).

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Poster

227. **Monoamine Transporters: Function and Modulation**

Location: Halls B-H

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Topic: B.05. Transporters

Support: NIH Grant MH076018 (MKH)

NIH Grant MH074700 (SRS)

Title: Noradrenergic axon terminals in the prefrontal cortex of mice heterozygous for the norepinephrine transporter show limited differences when compared to wild-type mice in plasmalemmal and cytoplasmic transporter localization and content of tyrosine hydroxylase

Authors: K. M. MARKLE[1], S. L. ERICKSON[1], *J. BALCITA-PEDICINO[1], M. K. HAHN[2], S. R. SESACK[1];
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Abstract:
Brainstem norepinephrine (NE) neurons that innervate the prefrontal cortex (PFC) modulate cognitive functions and attention. Malfunction of this NE projection contributes to depression and other disorders that are treated by blocking the NE transporter (NET). Interestingly, a mutation that fully knocks out the gene for NET results in neurochemical effects that mimic antidepressants. An important question is whether a heterozygous knockout (NET-het) similarly boosts NE levels by reducing NET protein on axonal membranes. The present study explored the ultrastructure of NE axons within the PFC of transgenic NET-het versus wild type (WT) mice. We hypothesized that NET-het mice would exhibit fewer NET-immunoreactive axons in the PFC as well as less total NET per profile. We expected these reductions to be accompanied by a redistribution of NET that preserved normal amounts of protein on the plasma membrane by drawing from the cytoplasm. We further hypothesized that detection of the rate-limiting synthetic enzyme tyrosine hydroxylase (TH) would increase in NE axons, particularly if normal plasmalemmal NET levels were not maintained. In a single cohort of 5 het and 5 WT mice, we examined PFC sections labeled by immunogold-silver for NET and found surprisingly no change in the density of labeled axons and no change in the distribution of NET to the membrane or cytoplasm. In adjacent sections double labeled for NET by gold and TH by immunoperoxidase, we similarly found no difference in the density of dually-labeled profiles. Within these axons, however, we found a significant reduction in the density of cytoplasmic gold for NET-het versus WT mice. There was no difference in the density of membrane gold, and so taken together, the proportion of total gold found on the plasma membrane was significantly increased for the NET-

het group. Finally, the percentage of NET-labeled axons that also exhibited TH was not different between NET-het and WT mice. These data suggest that PFC NE axons use homeostatic mechanisms (e.g. reduced NET degradation) to maintain normal levels of plasmalemmal NET regardless of whether total NET declines in each axon. The findings have important implications for understanding the capacity of the PFC NE system to adjust to partial loss of NET.

Disclosures: K.M. Markle: None. **S.L. Erickson:** None. **J. Balcita-Pedicino:** None. **S.R. Sesack:** None. **M.K. Hahn:** None.

Poster

227. **Monoamine Transporters: Function and Modulation**

Location: Halls B-H

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Topic: B.05. Transporters

Support: NIH Grants R25 OD010951 and K01 RR024471

Title: Effects of serotonin transporter deletion on microglial function

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Abstract: The past several decades have seen an emergence of studies providing evidence that the pathogenesis of depression cannot be solely explained by the monoamine theory, which holds that this devastating disorder is the result of a deficit of brain monoamine neurotransmission. Inflammation has been raised as an alternate theory. Recent studies have demonstrated that antidepressants have an anti-inflammatory effect on some immune cells, suggesting a possible alternate mechanism of action for antidepressants. Specifically, studies have shown that selective serotonin reuptake inhibitors (SSRIs), which target the serotonin transporter (SERT), have anti-inflammatory effects on microglia, the primary innate immune cells of the central nervous system. While a microglial cell line has been reported to express SERT, studies exploring whether this expression occurs *in vivo* are lacking. Determining whether SERT expression occurs in microglia and its possible role in the anti-inflammatory effects of SSRIs would further our understanding of how inflammatory processes may be involved in the pathogenesis of depression and/or the mechanism of action of antidepressants.
To this end, a mouse line expressing yellow fluorescent protein under the SERT promoter was

used to investigate expression of SERT in microglia. Primary microglia from SERT knockout (KO) mice and wild type (WT) mice were also used to explore the role of SERT deletion and pharmacologic blockade on microglial inflammatory responses.
Preliminary findings include evidence of SERT expression *in vitro* by the co-expression of SERT and the microglial marker Iba1 in primary microglial cell cultures. Studies are currently underway to further evaluate under which conditions, if any, *in vivo* SERT expression occurs. In addition, pharmacologic studies evaluating multiple SSRIs are ongoing to determine whether the anti-inflammatory effects seen thus far are due to action at SERT, which may be expressed on microglia, or due to off-target effects.

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Poster

227. **Monoamine Transporters: Function and Modulation**

Location: Halls B-H

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Topic: B.05. Transporters

Support: NIH Award MH096972

Pilot Award from the Vanderbilt Silvio O. Conte Center for Neuroscience Research

Title: A novel trans-synaptic neuroligin 2/serotonin transporter protein complex regulates serotonin signaling

Authors: *R. YE[1,4], M. QUINLAN[1], D. AIREY[1], H. IWAMOTO[1,4], H.-H. WU[4,5], C. JETTER[2], J. VEENSTRA-VANDERWEELE[1,2,3], P. LEVITT[4,5], R. D. BLAKELY[1,2,4];
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Abstract: Compromised serotonin (5-HT, 5-hydroxytryptamine) signaling has been implicated in multiple neuropsychiatric and neurodevelopmental disorders including depression, obsessive-compulsive disorder (OCD), anxiety and autism. The presynaptic 5-HT transporter (SERT) is the primary mechanism of extracellular 5-HT inactivation and is a major target of antidepressant medications. In an effort to identify novel regulators of 5-HT signaling, we implemented proteomic and genetic paradigms, efforts that converged on the synaptic cell adhesion gene neuroligin 2 (Nlgn2). Whereas NLGN2 protein appears to coordinate GABA signaling in the

forebrain, our profiling of midbrain RNA expression in recombinant inbred mice revealed that Nlgn2 gene expression strongly correlated with the expression of multiple genes required for 5-HT identity and signaling, including Fev (PET-1), Tph2, and Slc6a4 (SERT). These results converged with proteomic and co-immunoprecipitation studies which showed that NLGN2 forms stable, midbrain-specific protein associations with SERT. These findings have led us to hypothesize that NLGN2 makes critical contributions to both 5-HT and GABA mediated regulation of raphe neurons via a somato-dendritic SERT/5-HT1A/NLGN2/GABAA receptor complex. Our preliminary data using constitutive NLGN2 KO mice demonstrate that NLGN2 is required to maintain normal 5-HT levels, normal SERT protein expression, and performance on tasks sensitive to antidepressants. NLGN2 KO mice also displayed social avoidance as they backed away more frequently when confronted by another mouse in tube test. Our findings on the essential roles of NLGN2 in 5-HT signaling may relate to the rare gene variants in NLGN2 identified from subjects with neuropsychiatric disorders and raises the possibility that psychiatric disorders traditionally associated with altered 5-HT signaling may in some cases originate from perturbation of cell-adhesion mechanisms linking 5-HT and GABA signaling in the midbrain.

Disclosures: R. Ye: None. **M. Quinlan:** None. **D. Airey:** None. **H. Iwamoto:** None. **H. Wu:** None. **C. Jetter:** None. **J. Veenstra-VanderWeele:** None. **P. Levitt:** None. **R.D. Blakely:** None.

Poster

227. **Monoamine Transporters: Function and Modulation**

Location: Halls B-H

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Topic: B.05. Transporters

Support: Vanderbilt Silvio O. Conte Center for Neuroscience Research (NLB)

NIH Award MH078028 (RDB)

Institute for Psychiatric Neuroscience (RDB)

Title: The role of ongoing versus early-life IL-1R/p38 MAPK signaling in raphe neurons in the enduring effects of early-life stress

Authors: *N. L. BAGANZ[1,2], L. J. HARBOM[1], J. T. SMITH[1], R. D. BLAKELY[1,2,3];
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Abstract: Depression is the primary cause of disability worldwide and has long been linked to alterations in serotonin (5-HT) signaling, as well as perturbed immune system function. We are pursuing the hypothesis that depression may represent an inappropriate expression of basic physiological processes normally organized to modulate behavior in the context of immune system activation. Supporting this concept, patients diagnosed with depression often display higher levels of proinflammatory cytokines, such as interleukin-1β (IL-1β). Moreover, peripheral administration of IL-1β and other inflammatory agents produces depressive-like effects on behavior in rodents and humans, effects that can be attenuated or eliminated with antidepressant medications. Despite these long-standing observations, the cellular and molecular pathways by which immune system activation alters behavior remain ill defined. We propose that one of these pathways involves immune system signaling to, and through, regulatory pathways that insure proper 5-HT signaling. A prominent control point for 5-HT signaling is the presynaptic, antidepressant-sensitive 5-HT transporter (SERT, *SLC64A*). SERT activity can be rapidly increased by IL-1 receptor (IL-1R) activation *in vitro* by IL-1β through a p38 MAPK-dependent pathway. Additionally, i.p. injection of lipopolysaccharide (LPS) rapidly enhances synaptosomal 5-HT transport and 5-HT clearance. Finally, LPS also produces a depressive-like effect on behavior as assessed in the tail suspension (TST) and forced swim tests (FST), effects that are absent in IL-1R KO mice. Since early-life stress is a risk factor for the development of mood disorders in adults, and in animal models produces changes in adult immune system activation, we have implemented a maternal separation (MS) model to investigate whether the ensuing anxiety and depressive behavior of adult animals is dependent on IL-1R function and also whether such signaling involves early or later life (or both) modulation of CNS 5-HT signaling. Here we provide evidence that the anxiety- and depressive-like effects of MS observed in older animals are lost in IL-1R KO mice. Ongoing studies with conditional, raphe-specific elimination of IL-1R and p38 MAPK seek to determine whether CNS 5-HT neurons provide a critical link between early-life stress, the immune system and risk for depression.

Disclosures: N.L. Baganz: None. **L.J. Harbom:** None. **J.T. Smith:** None. **R.D. Blakely:** None.

Poster

227. **Monoamine Transporters: Function and Modulation**

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Topic: B.05. Transporters

Support: NIH Grant DA026530

NIH Grant DA027806

Hunkele Award, Duquesne Univ.

Title: Discovery of monoamine transporter ligands via virtual screening with the S1 substrate pocket of the serotonin transporter

Authors: L. M. GEFFERT[1], R. R. ROSS[1], T. L. NOLAN[1], B. J. KOLBER[2], J. D. MADURA[3], *C. K. SURRATT[1];
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Abstract: The relatively recent elucidation of an x-ray crystal structure for LeuT, a leucine transporter and bacterial homolog of the mammalian solute carrier 6 (SLC6) family, was a major advance toward understanding the 3-D structure of plasmalemmal monoamine transporter (MAT) proteins. Using LeuT as a template, a credible 3D computational model of the human serotonin transporter (SERT) has been developed using Molecular Operating Environment (MOE) software. Ligand docking simulations with the model identified binding pockets consistent with the SERT S1 and S2 sites known to bind both substrates and inhibitors. Structure-based virtual screening (VS) of the PubChem small molecule structural database using MOE and the primary substrate (S1) SERT pocket yielded 19 “hit” compounds. In vitro pharmacologic testing of the VS hits revealed four structurally unique compounds that inhibited substrate transport and displayed nanomolar binding affinity K_i values for at least one of the three plasma membrane MATs. The four compounds were characterized in vivo using C57/BL mice via the tail suspension test (TST), a measure of acute depression/anxiety. Two of the compounds significantly decreased mouse TST immobility times at moderate doses, an outcome that suggests antidepressant/anxiolytic effects in humans. It is hoped that the approach outlined above will provide a new avenue toward discovery of medications for treating depression, anxiety, chronic pain, addiction and other CNS-related disorders. By focusing on compounds of unique structural scaffold, the VS hit compounds may lead to drugs carrying fewer adverse effects. This study suggests that MAT computational models are useful for identification or development of lead compound pharmacotherapies, and to our knowledge is the first report of MAT inhibitor discovery using the S1 pocket as a VS tool.

Disclosures: L.M. Geffert: None. **R.R. Ross:** None. **T.L. Nolan:** None. **B.J. Kolber:** None. **J.D. Madura:** None. **C.K. Surratt:** None.

Poster

227. **Monoamine Transporters: Function and Modulation**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 227.11/E20

Topic: B.05. Transporters

Support: NIH Award MH094527 (RDB)

Title: Are the acute and chronic behavioral actions of serotonin selective reuptake inhibitors mediated solely by the serotonin transporter? Studies of drug action with the SERT M172 mouse

Authors: *A. G. NACKENOFF[1,2], A. B. MOUSSA-TOOKS[1], R. D. BLAKELY[1,3];
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Abstract: Depression is one of the most common and burdensome disorders worldwide. The most widely prescribed antidepressants are serotonin (5-HT) selective reuptake inhibitors (SSRIs), believed to provide therapeutic benefit by antagonizing the 5-HT transporter (SERT) and elevating extracellular 5-HT levels. Significant, indirect evidence supports this conclusion, although multiple SSRIs have been found to interact with a sizable number of non-trivial targets at physiologically relevant concentrations. Several of these targets, when manipulated pharmacologically or genetically, can induce some of the same biochemical and behavioral effects associated with SSRI administration, raising the question whether all of the effects of SSRIs are 5-HT mediated. In order to separate the 5-HT/SERT dependent effects from the non-SERT effects of SSRI administration, we developed a transgenic mouse line that expresses a point mutation in the SERT SSRI binding site, converting an Ile at amino acid 172 to Met (SERT M172) (Thompson et al, PNAS 2011). The M172 substitution is benign with respect to SERT protein expression and 5-HT transport activity in vitro and in vivo, but confers a marked reduction in sensitivity (10-1000 fold) to many SERT antagonists. In our prior studies, where SERT M172 was expressed on a 129S6/S4 background, WT mice on the same background demonstrated either meager or anomalous responses to SSRIs in classical, acute tests of antidepressant action (tail suspension test and forced swim test). Although insensitivity was documented for citalopram and fluoxetine in biochemical, physiological, and behavioral assays, deeper analyses of SSRI action, particularly with chronic administration, required moving the M172 variant to a more favorable genetic background. After producing SERT M172 mice congenic on a C57BL/6J background, we repeated our initial characterization studies, validating prior observations that the variant supports normal synaptosomal 5-HT uptake kinetics but disrupts the ability of multiple SSRIs to inhibit 5-HT uptake. Moreover, these SERT M172 mice permitted a clear demonstration that SERT, and enhanced extracellular 5-HT, is essential for the immobility reducing behavioral actions of acute administration of citalopram and fluoxetine. Our ongoing studies aim to evaluate the requirement of SERT interactions for the effects of chronic antidepressant action, as assessed in behavioral, biochemical and stem cell analyses. Additionally, RNA profiling of SSRI-treated SERT M172 animals will provide an opportunity to elucidate gene and protein expression networks dependent and independent of SSRI-modulated 5-HT signaling.

Disclosures: A.G. Nackenoff: None. **A.B. Moussa-Tooks:** None. **R.D. Blakely:** None.

Poster

227. **Monoamine Transporters: Function and Modulation**

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Topic: B.05. Transporters

Support: MH093320

MH064489

NARSAD

UL1RR025767

Title: Mechanisms contributing to lack of antidepressant efficacy in juveniles and adolescents

Authors: *N. MITCHELL[1], W. OWENS[1], R. HORTON[1], M. VITELA[1], G. GOULD[1], W. KOEK[2,3], L. DAWS[1,3];
[1]Physiol., [2]Psychiatry, [3]Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: Depression is a major health problem for which most patients are not effectively treated. This problem is further compounded in children and adolescents where only two antidepressant drugs are currently approved for clinical use. Both are selective serotonin reuptake inhibitors (SSRIs), which are often less therapeutically efficacious in this young population compared to adults. Consistent with clinical literature, we found that antidepressant-like effects of SSRIs in mice aged 21 days post-partum (P21, juvenile) was reduced relative to adult mice. The increase in extracellular 5-HT following SSRI administration is thought to trigger downstream events required for therapeutic efficacy. Thus, our data raise the possibility that transporters capable of 5-HT uptake other than SERT may be present in disproportionately higher levels than SERT during juvenile and adolescent periods thereby preventing extracellular 5-HT from climbing to therapeutically relevant levels. We found that SERT expression in hippocampus of juvenile and adolescent (P28) mice is lower compared to adult mice. Conversely, in juveniles and adolescents, the density of [^{3}H]decynium-22 (D22) binding sites, which include organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT), was greater than that of binding sites for the SERT ligand, [^{3}H]citalopram. Western blot analysis using specific antibodies revealed that increased [^{3}H]D22 binding was

most likely driven by increased PMAT expression in young mice relative to adults.
Studies are underway to assess the antidepressant-like effects of blocking PMAT (and/or OCTs) in juvenile and adolescent mice, as well as the ability of D22 to inhibit 5-HT clearance in these young mice. Because PMAT and OCTs can take up serotonin from extracellular fluid, these transporters may limit the therapeutic efficacy of SSRIs, providing a mechanistic basis for poor treatment response to SSRIs particularly
in juveniles and adolescents.

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Poster

228. **Dopamine Transporters: Modulation and Disease**

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Program#/Poster#: 228.01/E22

Topic: B.05. Transporters

Support: Danish Medical Research Council

University of Copenhagen BioScaRT Program of Excellence

Lundbeck Foundation

Title: Unique neuro-adaptations of the dopaminergic synapse in DAT mutant mice with altered cocaine-, food-, and sugar-reinforced operant behavior

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Abstract: Dopamine has an important modulatory role in the central nervous system controlling motor activity, cognition and reward mechanisms. Impairment of dopaminergic function is associated with several neuropsychiatric diseases. Re-uptake from the extracellular compartment by the dopamine transporter (DAT) is crucial for homeostatic transmitter levels. In the basal ganglia, dopaminergic modulation of the direct striatonigral and the indirect striatopallidal pathways are critical in reward learning and drug addiction.

This unique mouse model was originally generated as an in vivo model in which DAT contains a substitution in the extreme C-terminus that promotes a higher constitutive internalization of an otherwise functional transporter. This subtle mutation in DAT reduces DAT levels.
In order to analyze the mutant mice for in vivo neuro-adaptations of the dopaminergic synapse, we measured total striatal dopamine levels and clearance from the extracellular space. In addition, we assessed striatal D1 receptor, and D2 receptor distribution as well as D2 autoreceptor function. Behaviorally, we investigated the operant self -administration of the mutant mice when reinforced by cocaine, food, and sugar.
We found a decreased level of total striatal DA, and a decrease in D2 receptors but no change in D1 receptor levels in mutant mice. In addition, in voltammetry experiments mutant mice had a prolonged clearance time of extracellular DA than wild-type mice.
Mutant mice learned cocaine-reinforced operant behavior similar to wild-type mice during naïve acquisition but earned significantly less cocaine reinforcers than their wild-type littermates in a dose-response FR1 schedule of reinforcement. In contrast to this, mutant mice showed significantly higher response rates for liquid food and sugar under FR1 and for liquid food under PR schedules.
Our results suggest that disruption of PDZ-protein interactions has profound influence on the role of DAT in regards to cocaine-, food, and sugar-induced reinforcing behaviour and underlines the importance of DAT in the regulation of dopaminergic homeostasis.

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Poster

228. **Dopamine Transporters: Modulation and Disease**

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AA007565

AA014445

Title: Increases in rapid nucleus accumbens dopamine signaling, and cocaine and methylphenidate potency in social isolation reared rats

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Abstract: Social isolation rearing (SI) is a model of early life stress that results in neurobiological alterations that lead to increased vulnerability to addiction-like behaviors. Striatal dopamine circuitry is a key mediator of behavioral changes that occur due to long-term stressors. These changes are characterized by increased dopamine metabolite tissue content, midbrain dopamine neuron burst firing, and increased dopamine overflow to psychostimulants in SI versus group reared (GH) animals. Because of the documented increase in psychostimulant effects in SI animals relative to GH, we aimed to assess the potency of two structurally dissimilar dopamine uptake inhibitors, cocaine and methylphenidate (MPH). Long-Evans rats were either group housed (GH; 4/cage) or SI (1/cage) from weaning into early adulthood [postnatal day (PD) 28-77] and neurochemical changes were assessed at PD 77 using fast scan cyclic voltammetry in brain slices. Voltammetric detection of dopamine allows for the assessment of release and uptake kinetics as well as assessing shifts in drug potency at the dopamine transporter (DAT). We show that SI rats, relative to GH, show marked increases in electrically-evoked dopamine release and uptake. Increases in uptake rates are mediated by increases in DAT levels in SI animals, which have been confirmed with western blot hybridization. Surprisingly, the ability of MPH, but not cocaine, to inhibit DA uptake is significantly greater in SI animals relative to GH. Although cocaine effects on uptake were not greater in isolates, we did observe larger increases in cocaine, but not MPH induced release after SI rearing.
The differential effects of MPH and cocaine could be due to structural differences between the two compounds. MPH is structurally similar to the releaser AMPH, which has previously been demonstrated to be more behaviorally potent in the DAT transgenic overexpressing mice as well as SI animals. Further, these data indicate that the potency of cocaine at the DAT is likely not involved in the increased cocaine-induced dopamine overflow that has been shown previously in SI animals, but rather due to the increased releasing effects of cocaine, possibly through interactions at release related machinery.

Disclosures: J.T. Yorgason: None. **E.S. Calipari:** None. **M. Ferris:** None. **B.A. McCool:** None. **J.L. Weiner:** None. **S.R. Jones:** None. **N.J. Alexander:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: NIH Grant DA13975

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Title: PIP2 regulation of amphetamine-induced dopamine efflux and behaviors

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Abstract: Amphetamine (AMPH) abuse is a major societal problem with severe psychological and physiological consequences for the abusing individual. The major molecular target of AMPH is the dopamine (DA) transporter (DAT), a member of the Na^+/neurotransmitter symporter family. AMPH's addictive properties are mediated by its ability to increase extracellular concentrations of DA by reversing DAT function and, as a consequence, causing DA efflux into the synaptic cleft. Here, we show that the N-terminus of the human DAT (hDAT) interacts electrostatically with phosphoinositol-4,5-bisphosphate (PIP_2; a key signaling molecule known to interact with and regulate ion channels and transporters) and that this interaction regulates AMPH-induced DA efflux, while hDAT uptake and DA clearance functions are unaffected. Using computational modeling of the hDAT N-terminus/plasma membrane interaction to guide our experiments, we have identified key residues on the N-terminus that directly interact with PIP_2, Lys3 and Lys5. Mutation of these residues to either Ala or Asn disrupts the interaction between the hDAT N-terminus and PIP_2 *in silico*, and *in vitro*, severely impairing the ability of the hDAT to efflux DA in response to AMPH. Importantly, DA uptake is unaffected in these mutants, suggesting an asymmetric mechanism for selectively regulating one aspect of the hDAT transport cycle. Finally, using a behavioral model we developed in *Drosophila melanogaster* (namely, locomotion), we determined that the N-terminus interaction with PIP_2 regulates the ability of AMPH to induce behaviors. These findings have implications for developing efflux-selective therapies for AMPH abuse, while preserving endogenous DA uptake function. To our knowledge, this is the first demonstration that the interaction of hDAT with membrane lipids regulates AMPH-induced DA efflux, and that this interaction has profound effects on organismal behavior. **These authors contributed equally to this work.

Disclosures: A.N. Belovich:** None. **P.J. Hamilton**:** None. **C. Saunders:** None. **G. Khelashvili:** None. **H. Weinstein:** None. **H. Sitte:** None. **H.J.G. Matthies:** None. **A. Galli:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: NIDA Grant DA026947

NINDS Grant NS071122

Title: Intracellular S(+)-Methamphetamine decreases dopamine-dependent inward current via dopamine transporter

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Abstract: Dopamine (DA) transporter (DAT) regulates the spatial and temporal dimensions of brain dopaminergic neurotransmission. DAT is implicated in neuropsychiatric conditions such as schizophrenia, Attention-deficit hyperactivity disorder, Parkinsonism and drug addiction. Drugs of abuse like amphetamine (AMPH) and methamphetamine (METH) are DAT substrates. They increase DA levels in the synapse by competing with DA for uptake as well as by inducing reverse transport of DA via DAT albeit by poorly understood mechanism. Although DA's chemical structure is similar to that of AMPH and METH enantiomers, DAT responses to these drugs are fundamentally different. We have shown that as compared to AMPH, systemic METH increases the amplitudes and duration of extracellular dopamine 200 times more than AMPH in the nucleus accumbens. In the present study we will examine the underlying molecular mechanism of these differences. We used voltage-clamp electrophysiology in mammalian cells expressing Yellow fluorescent-tagged DAT to examine the hypothesis that differences in chemical structure or chirality of substrates influence the nature of DAT-mediated inward currents via a use-dependent mechanism. Our data suggest that S(+)-METH elicits a DAT-mediated inward current larger than that of DA, S(+)-AMPH, racemic mixture of METH (S(+), R(-) isomers) or R(-)-METH alone. Thus, R(-)-METH may be a DAT partial agonist which decrease DAT current elicited by S(+)-METH, the more effective stereoisomer. Next, we

determined DAT-mediated inward current when the drugs are individually dialyzed into the cell via the patch pipette. In the absence of extracellular substrate, the intracellular drug application via the patch-pipettes produced lesser inward current that was not significantly different amongst the tested compounds. Lastly we studied the nature of DA-induced inward current (a measure of DA uptake) when either S(+)-METH or S(+)-AMPH are dialyzed into the cells via the patch pipette. Compared to S(+)-AMPH, the intracellular S(+)-METH reduced the magnitude of DA-induced inward current when DA is applied extracellularly. Ongoing experiments will examine the effects of these compounds in DA neurons and their effect on the firing activity of DA neurons. Findings from our experiments will provide new research tools to study DAT and potentially determine the mechanistic differences between structurally similar DAT substrates required for the development of effective and economically viable therapeutic approaches for psychostimulant addiction.

Disclosures: K. Saha: None. **D. Angoli:** None. **P. Davari:** None. **L. Villarroel:** None. **H. Khoshbouei:** None. **S. Goodwin:** None.

Poster

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Title: Methamphetamine affects the plasma membrane mobility of the dopamine transporter via a calcium-dependent mechanism

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Abstract: Neurotransmitter reuptake by transporters is a major mechanism for terminating synaptic transmission. The human dopamine transporter (hDAT) is one of the main targets for psychostimulants, and is critical to DA homeostasis in the brain. We previously reported that at maximally effective concentrations, METH released significantly more calcium from internal stores compared with AMPH. The effect of AMPH and METH on intracellular calcium mobilization was thapsigargin-sensitive and hDAT-dependent. Pretreatment of the cells with hDAT antagonist blocked the response. Our current findings implicate elevated levels of calcium in regulating DAT diffusion and membrane microdomain association. Using fluorescence recovery after photobleaching (FRAP) we found that METH decreased the diffusion rate (D) and mobile fraction (Mf) of hDAT. Treatment with thapsigargin, which releases and prevents reuptake of calcium from the ER, mimics the METH mediated decrease in D and Mf of hDAT. Treatment with BAPTA, a calcium chelator, restores DAT diffusion and Mf in the presence of METH. Additionally, treatment with cytochalasin D, which inhibits actin polymerization and induces depolymerization of actin filaments, not only restores hDAT diffusion and mobile fraction in the presence of METH but also increases DAT diffusion in control conditions. These preliminary data are consistent with the interpretation that METH-induced, DAT-mediated elevation in intracellular calcium in DAT-expressing cells affects the actin cytoskeleton, decreasing the mobility of hDAT.

Disclosures: J.S. Goodwin: None. **K. Saha:** None. **P. Davari:** None. **L. Villarroel:** None. **H. Khoshbouei:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: DA026947

Title: σ-1 receptor regulates methamphetamine inhibition of substrate uptake via dopamine transporter

Authors: *D. O. SAMBO[1], M. LIN[1], B. BLOUGH[2], D. ANGOLI[1], H. KHOSHBOUEI[1]; [1]Univ. of Florida, Gainesville, FL; [2]RTI Intl., Research Triangle Park, NC

Abstract: The principle mechanism for terminating dopaminergic signaling is the reuptake of dopamine via the dopamine transporter (DAT). The activity of this Na^+/Cl^- symporter determines the intensity and duration of dopamine neurotransmission in the brain. The transporter is implicated in a variety of neurological disorders and is one of the primary targets for psychostimulants such as cocaine and methamphetamine (METH). METH is a highly addictive drug that competes with dopamine at the transporter and induces dopamine efflux via DAT. Past studies indicate that self-administration of METH causes upregulation of the σ-1 receptor, a chaperone protein at the endoplasmic reticulum-mitochondria interface, in midbrain regions. Upon activation, this non-opioid receptor translocates to the plasma membrane where it has been shown to modulate the activity of various receptors and channels. Using co-immunoprecipitation and fluorescence resonance energy transfer (FRET) we have found that the DAT and σ-1 receptor interact at the plasma membrane, and that this interaction is potentiated by treatment with METH. In this study, we investigated the functional consequence of the DAT/σ-1 receptor interaction on the activity of the transporter. Through live cell imaging in DAT-expressing cells, we monitored uptake of a fluorescent substrate of DAT, ASP^+, when the σ-1 receptor is overexpressed. Our preliminary data suggests that overexpression of σ-1 receptor increases the rate of DAT-mediated substrate uptake both at baseline and when treated with METH. Consistent with the literature, METH treatment decreased DAT-mediated substrate uptake. In this study we found that overexpression of σ-1 receptor in DAT cells blocked the METH induced inhibition of substrate uptake. These results suggest that the upregulation of the σ-1 receptor can modulate the activity of the transporter both at baseline and when exposed to METH. Ongoing experiments examine the underling mechanism of σ-1 receptor regulation of DAT activity in dopaminergic neurons. Because METH self-administration increases σ-1 receptor levels in brain regions with the highest number of DAT positive terminals, understanding the mechanism of σ-1 receptor regulation of DAT activity may reveal novel therapeutic approaches for the treatment of METH addiction.

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

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: NIH DA007595

NIDA Intramural Research

Title: Exploring the association of the dopamine transporter with the sigma-1 receptor

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Abstract: The dopamine transporter (DAT) clears dopamine (DA) released from presynaptic terminals and plays an important role in regulating DA neurotransmission. The sigma receptor was initially proposed as a subtype of opioid receptors, and sigma ligands were shown to inhibit midbrain dopamine neuronal firing and modulate dopamine release in earlier pharmacological studies (Walker et al., Pharmacol Rev 1990). Recent progress has demonstrated that the sigma-1 receptor (Sig-1R) is a ligand-modulated molecular chaperone (Hayashi and Su, Cell, 2007) capable of interacting with a variety of membrane channels and receptors (Kourrich et al., Trends Neurosci, 2012). To explore the potential association between Sig-1R and plasmalemmal neurotransmitter transporters, we performed co-immunoprecipitation studies in transfected cells. Sig-1R co-immunoprecipitated with DAT, but not with the excitatory amino acid transporter EAAT2. We then generated truncation mutants of Sig-1R to dissect the necessary domains involved in this association. Deletion of the short N-terminus or a major portion of the C-terminus of Sig-1R did not abolish its co-immunoprecipitation with DAT, suggesting that the two transmembrane domains of Sig-1R are likely crucial in mediating its association with DAT. Intriguingly, a splice variant of Sig-1R which lacks the C-terminus exhibited enhanced association with DAT, compared with the full-length Sig-1R. Preliminary data also showed that the association of Sig-1R and DAT could be regulated by Sig-1R ligands, as treatment with the agonist (+)pentazocine reduced the amount of Sig-1R associated DAT. Whether the dynamic association of the DAT with Sig-1R can affect transporter surface expression and modulate DA neurotransmission is currently under investigation.

Disclosures: W.C. Hong: None. **S.G. Amara:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Title: Electrophysiological and amperometric evidence that modafinil blocks the dopamine uptake transporter to induce behavioral activation

Authors: *N. B. MERCURI, M. FEDERICI, C. LATAGLIATA, F. RIZZO, R. NISTICÒ, S. PUGLISI-ALLEGRA;
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Abstract: Although the wake-promoting drug modafinil has been shown to bind quite exclusively to the dopamine transporter (DAT), its action in the brain has been thought to be partially independent from the facilitation of the dopaminergic signals.
Here we used electrophysiological and amperometric techniques to investigate the effects of modafinil on the dopaminergic neurons of the substantia nigra *pars compacta* (SNpc) and on the synaptic release of dopamine in the dorsal striatum from sliced tissue of wild-type and cocaine-insensitive genetically modified mice (DAT-CI). Moreover, we examined the consequences of modafinil administration on the locomotor behavior of wild-type and DAT-CI mice.
In *in vitro* experiments, modafinil inhibited the spontaneous firing discharge of the dopaminergic neurons. More consistently, it potentiated firing inhibition and the membrane responses caused by exogenously applied dopamine on these cells. Furthermore, it augmented the stimulus-evoked outflow of DA in the striatum. Noteworthy, modafinil caused locomotor activation in wild-type mice. On the other hand, neither the electrophysiological nor the behavioral effects of modafinil were detected in DAT-CI animals.
These results demonstrate that modafinil potentiates brain dopaminergic signals via DAT inhibition by acting at the same binding site of cocaine. Therefore, this mechanism of action explains most of the pharmacological properties of this compound in the clinical setting.

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Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: DA013975

DA012408

Title: Dopamine transporter coding variant Ala559Val associated with attention deficit hyperactivity disorder impairs dopamine transporter trafficking and function

Authors: E. A. BOWTON[1], C. SAUNDERS[2], D. SAKRIKAR[2], R. D. BLAKELY[2], H. J. G. MATTHIES[1], A. GALLI[1], *K. ERREGER[1];
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Abstract: Alterations in components of brain dopamine (DA) signaling and homeostasis have been implicated in the pathophysiology of neuropsychiatric disorders including attention deficit hyperactivity disorder (ADHD). The human dopamine transporter (DAT) variant with the nonsynonymous single nucleotide polymorphism Ala559Val ("hDAT A559V") was found in two male siblings with ADHD and had previously been reported in one female with bipolar disorder. When heterologously expressed, hDAT A559V exhibits anomalous non-vesicular DA release (DA efflux) that mimics the actions of amphetamine (AMPH)-like psychostimulants. AMPH acts by promoting DA efflux through DAT and recruiting signaling pathways that regulate DAT function, including protein kinase C (PKC) activation and increased phosphorylation of DAT. Importantly, AMPH has also been shown to cause trafficking leading to internalization of DAT. Previous data suggest that intracellular AMPH accumulation is required for AMPH-induced DAT trafficking, possibly due to the ability of AMPH to increase intracellular Ca2+ levels and stimulate Ca2+-dependent kinases including PKC.
Here, we show that while AMPH induces DAT internalization in hDAT-expressing cells, AMPH has no effect on DAT trafficking in hDAT A559V cells. Moreover, AMPH fails to elicit DA efflux in hDAT A559V cells. However, both AMPH-induced internalization and AMPH-induced DA efflux are restored by 1) mutating N-terminal serines of hDAT A559V to alanine prevent phosphorylation or 2) pharmacologically inhibiting PKCβ activity, suggesting that phosphorylation of hDAT A559V N-terminal serines by PKCβ may support anomalous DA efflux activity of hDAT A559V. AMPH uptake was investigated at 10 nM AMPH (a concentration sufficient to induce AMPH uptake and DA efflux in hDAT cells) to avoid high nonspecific signals observed at larger AMPH concentrations. Under these conditions, AMPH is not transported by hDAT A559V, indicating that hDAT A559V is either unable to uptake AMPH or has a shifted apparent affinity for AMPH uptake compared with hDAT. Importantly, direct intracellular delivery of AMPH in hDAT A559V cells restores DAT internalization to levels similar to that seen in hDAT cells treated with AMPH. These data suggest that PKCβ-dependent phosphorylation of the hDAT A559V N-terminus impairs transport of AMPH, thereby preventing AMPH from eliciting its intracellular effects that subsequently result in DA efflux and in DAT internalization.
These results demonstrate how genetic variation in DAT coding regions can impact transporter trafficking and function, as well as potentially contribute to altered DA neurotransmission in disease states.

Disclosures: E.A. Bowton: None. **K. Erreger:** None. **D. Sakrikar:** None. **H.J.G. Matthies:** None. **R.D. Blakely:** None. **A. Galli:** None. **C. Saunders:** None.

Poster

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Title: De novo mutation in the dopamine transporter gene associates dopamine dysfunction with autism spectrum disorder

Authors: *P. J. HAMILTON[1], N. G. CAMPBELL[1], S. SHARMA[2], K. ERREGER[2], F. HERBORG HANSEN[5], C. SAUNDERS[3], A. N. BELOVICH[3], E. H. COOK, Jr.[6], U. GETHER[5], H. S. MCHAOURAB[2], H. J. G. MATTHIES[2], J. S. SUTCLIFFE[4], A. GALLI[2];
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Abstract: De novo genetic variation is an important class of risk factors for autism spectrum disorder (ASD). Recently, whole exome sequencing of ASD families has identified a novel de novo missense mutation in the human dopamine (DA) transporter (hDAT) gene, which results in a Thr to Met substitution at site 356 (hDAT T356M). The DAT is a presynaptic transmembrane protein that regulates dopaminergic tone in the central nervous system by mediating the high-affinity re-uptake of synaptically released DA, making it a crucial regulator of DA homeostasis. Here, we report the first functional, structural, and behavioral characterization of an ASD-associated de novo mutation in the hDAT. We demonstrate that the hDAT T356M displays anomalous function, characterized as a persistent reverse transport of DA (substrate efflux). Importantly, in the bacterial homolog leucine transporter, substitution of A289 (the homologous site to T356) with a Met promotes an outward-facing conformation upon substrate binding. In the substrate-bound state, an outward-facing transporter conformation is a required for substrate efflux. In Drosophila melanogaster, expression of hDAT T356M in DA neurons lacking Drosophila DAT leads to hyperlocomotion, a trait associated with DA dysfunction and ASD. Taken together, our findings demonstrate that alterations in DA homeostasis, mediated by aberrant DAT function, may confer risk for ASD and related neuropsychiatric conditions.
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Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: DA024275

DA026721

DA032910

DA035552

Title: Identification and characterization of recognition binding sites on human dopamine transporter involved in HIV-1 Tat-mediated disruption of dopamine transport

Authors: *N. M. MIDDE[1], X. HUANG[2], A. M. GOMEZ[1], C.-G. ZHAN[2], J. ZHU[1];
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Abstract: HIV-1 Tat protein impairs dopamine (DA) neurotransmission through inhibiting DA transporter (DAT) function. Based on the predictions of computational modeling and simulations, we have recently demonstrated that mutation (Y470H) of human DAT (hDAT) attenuates Tat-induced inhibition of DA transport. In the present study, we characterized the pharmacological profiles of two other mutations (Y88F and K92M) in hDAT for Tat binding. Compared to wild type hDAT (WT hDAT), the Vmax for DA uptake in CHO cells transfected with these mutants was differentially decreased: Y470H > K92M > Y88F without changes in Km values. Further, Y88F and K92M displayed decreased potency of DA substrate and increased potency of cocaine, WIN35,428, and GBR12909 for inhibition of DA uptake. These results suggest that the identified residues may not overlap with the binding sites in hDAT for

DA substrate but are critical for the interaction of these inhibitors with DAT. In addition, exposure to recombinant Tat1-86 decreased Vmax by 38% and 18% in WT hDAT and both Y88F and K92M, respectively, suggesting that these residues are important for HIV-1 Tat-induced inhibition of dopamine transport. Furthermore, Y88F and K92M altered zinc-mediated modulation of WIN35,428 binding and enhanced basal DA efflux compared to WT hDAT. Determining this potential mechanism underlying Tat-induced transporter conformational transitions is an important ongoing study. Collectively, these results provide additional information to understand the functional relevance of identified residues for Tat binding on hDAT and aid in developing compounds that can specifically block the functional binding of Tat to hDAT.

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Poster

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NSFC 31123002

Title: GDNF/Ret signaling regulates dopamine transporter trafficking via guanine nuclear exchange factor Vav2

Authors: *C. ZHAO, S. ZHU, J. ZHOU;
Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China

Abstract: The dopamine transporter (DAT) plays a key role in controlling the dopaminergic signaling and dopamine homeostasis via its dopamine reuptake activity. Here we found that glial cell line-derived neurotrophic factor (GDNF) reduced DAT activity in the nucleus accumbens by inducing the DAT endocytosis. In this process the association between GDNF receptor Ret and a Rho family GEF Vav2 is critical. GDNF-dependent phosphorylation of Vav2 modulated the internalization of Ret and DAT, thereby regulating the dopamine homeostasis. Moreover, Vav2 KO mice showed delayed response to cocaine in the addictive behavior. These results suggest that GDNF modulates the dopamine transmission via regulating DAT internalization.

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Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: T32 MH 16804-31

Title: Gβγ protein activation promotes dopamine efflux trough the Dopamine Transporter

Authors: *J. GARCIA-OLIVARES[1], S. G. AMARA[1], G. E. TORRES[2];
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Abstract: Proper function of reward circuitries in the brain requires that the presynaptic dopamine transporter (DAT) efficiently recaptures dopamine (DA). DAT function can be regulated by many intracellular mechanisms including phosphorylation, ubiquitination, and protein-protein interactions. Recently, our laboratory reported a novel mechanism describing the regulation of DAT by G-proteins. Gβγ subunits bind DAT, and upon activation decrease DA uptake. We are now exploring whether activation of Gβγ subunits also affect DA efflux in an amphetamine-dependent or -independent manner. Using a radiolabeled efflux assay, we measured DA efflux in HEK-293 cells stably expressing DAT that were preloaded with [H3]-DA. Activation of Gβγ with the cell permeant peptide mSIRK resulted in a dose-dependent release of DA. This effect was blocked by cocaine and GBR12935 demonstrating that Gβγ activation promotes DA efflux through DAT. Similarly, amperometric recordings demonstrated that mSIRK activation of Gβγ also induced DA release in HEK-293-DAT cells. Taken together, our results suggest that DA efflux through DAT occurs by a G protein-dependent process and this novel mechanism might have important implications in the actions of amphetamine.

Disclosures: J. Garcia-Olivares: None. **S.G. Amara:** None. **G.E. Torres:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Support: alpha-syn NS071122

Title: Sumoylation modulates Alpha synuclein interaction with the Dopamine Transporter

Authors: *E. CARTIER[1], H. KHOSHBOUEI[2];
[2]Neurosci., [1]Univ. of Florida, Gainesville, FL

Abstract: Sumoylation is a posttranslational modification by which small ubiquitin-like modifiers (SUMO) are covalently conjugated to target proteins. Sumoylation is highly dynamic and reversible as SUMO specific proteases can rapidly remove SUMO from their substrates. The specific SUMO conjugase, UBC9, sumoylates target proteins. Conversely, the SUMO Interacting Domains (SIM) of non-sumoylated proteins can recognize conjugated SUMO. SUMO modification of a given protein has been shown to create or abolish binding interfaces that can regulate trafficking, protein-protein interactions, protein aggregation and solubility. Recent reports suggest that alpha-synuclein, a synaptic protein implicated in multiple neurodegenerative diseases and drug addiction, is sumoylated in two specific residues. Alpha-synuclein is one of DAT's interacting partners that directly associate with the C-terminus of the transporter. Confocal microscopy and biochemical data from our lab have demonstrated that alpha-synuclein and dopamine transporter interact at the plasma membrane. Whole-cell patch clamp electrophysiology results suggest that alpha-synuclein over-expression induces a transporter mediated inward chloride current and decreases DAT-mediated substrate uptake. DAT molecule contains a number of putative SIMs that might be critical in order to regulate DAT interaction with sumoylated alpha-synuclein. In this study we used co-immunoprecipitation (Co-IP) and biotinylation assays to examine whether DAT interacts with SUMO (SUMO-1 and/or 2), and whether sumoylation affects DAT/alpha-synuclein interaction. Our preliminary data suggest that DAT associates with the SUMO conjugase UBC9 and appears to be sumoylated. In addition, in the striatum, HEK and MN9D cells expressing DAT, we found a strong interaction between DAT and SUMO-1 which is consistent with the existence of one or more SIM in the DAT structure. Moreover, sumoylation appears to reduce the association between DAT and alpha-synuclein, increasing the expression of DAT at the plasma membrane. Our ongoing confocal microscopy will examine whether sumoylation regulates DAT/alpha-synuclein interaction in living cells. The functional assays will demonstrate the functional significance of these observations.

Disclosures: E. Cartier: None. **H. Khoshbouei:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 228.15/E36

Topic: B.05. Transporters

Support: NS071122

NS071122S1

Title: Alpha-synuclein regulates trafficking of dopamine transporter

Authors: *B. R. BUTLER[1], E. CARTIER[2], D. ANGOLI[2], H. KHOSHBOUEI[2];
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Abstract: The dopaminergic system regulates several brain functions including motor, behavior and reward. The dopamine transporter (DAT) is a 12 multi-pass transmembrane protein, which regulates the magnitude and timing of dopaminergic signaling by re-uptake of excess dopamine from the synapse as well as efflux of dopamine into the synapse. Dysregulation of DAT function has been implicated in neurodegenerative, neuropsychiatric disorders and drug addiction. DAT directly or indirectly interacts with multiple proteins. These protein partners have shown to alter the DAT-mediated substrate uptake, efflux and/or the trafficking of the transporter. Alpha-synuclein is one of DAT's binding partners that directly interact with the C-terminus of the transporter. Confocal Microscopy and molecular biochemistry data from our lab have demonstrated that alpha-synuclein and dopamine transporter interact at the plasma membrane. Whole-cell patch clamp electrophysiology results suggest that alpha-synuclein over-expression induces a transporter mediated inward chloride current and decreases DAT-mediated substrate uptake. In the current studies we examined whether alpha-synuclein influences DAT trafficking and when the transporter is exposed to 10 uM amphetamine or 1 uM phorbol ester (PMA). We used live-cell confocal microscopy and biotinylation assay to examine the relative level of DAT at the surface membrane when alpha-synuclein is over-expressed, as compared to the absence of intracellular alpha-synuclein. Our preliminary results show that alpha-synuclein stabilizes the transporter at the surface membrane and decreases amphetamine-induced DAT internalization. Currently, we are examining these findings in alpha-synuclein wild-type, knockout and overexpressing neurons. These preliminary findings support the hypothesis that pathological levels of alpha-synuclein can alter the biology of DAT and thus dopamine transmission in the brain. Research funded by NS071122, NS071122S1.

Disclosures: B.R. Butler: None. **E. Cartier:** None. **D. Angoli:** None. **H. Khoshbouei:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Program#/Poster#: 228.16/E37

Topic: B.05. Transporters

Support: ARRA-funded NIH grant DA07595

Title: Protein-kinase c mediated internalization of the dopamine transporter

Authors: *S. M. UNDERHILL, S. G. AMARA;
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Abstract: The dopamine transporter (DAT) clears neurotransmitter from the extracellular space and serves as an important regulator of signal amplitude and duration at sites of dopamine release. In both DA neurons and transfected cell lines, the activation of several different intracellular signaling pathways has been observed to modulate DAT activity by regulating the trafficking of carriers to and from the cell surface. PKC signaling has been the most extensively studied of these mechanisms for regulating transporter cell surface density, and it has been well-established that acute activation of PKC by phorbol esters facilitates internalization of the transporter in a variety of model systems. However, the physiological stimuli and cell-surface receptor systems that activate PKC and potentially regulate the DAT in dopamine neurons within the mammalian brain remain elusive. In studies presented here we sought to identify the endogenous GPCRs coupled to PKC-activation that regulate DAT internalization in DA neurons. Because three of the five muscarinic acetylcholine receptor isoforms are coupled to PKC activation and one of these, M1, is found in dopamine neurons, we hypothesized that DAT internalization could be mediated by PKC-coupled M1 muscarinic acetylcholine receptor stimulation in dopamine neurons.
We found that stimulation of M1 receptors with carbachol in midbrain cultures decreased the ability of these cells to transport dopamine through DAT. The M1-specific antagonist, pirenzepine, blocked these effects while the M3 antagonist, DAU 5884, had no effect, indicating the receptor subtype responsible for modulation in dopamine neurons is most likely the M1 isoform. A PKC inhibitor, bisindolylmaleimide, blocked the effects of carbachol stimulation on dopamine uptake supporting a role for PKC in DAT internalization. Furthermore, as shown previously for PKC-induced internalization, downregulation of the DAT was clathrin- and dynamin-dependent. In acute midbrain slices, biotinylation of cell-surface proteins revealed the loss of dopamine transport mediated by M1 receptor stimulation was, indeed, due to loss of membrane expression of the DAT. These data indicate that stimulation of cholinergic pathways can lead to modulation of dopamine through internalization of the DAT.

Disclosures: S.M. Underhill: None. **S.G. Amara:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: NIH Grant DA026947

NIDA-IRP

NIH Grant DA12408

Title: Voltage-dependent regulation of dopamine transporter trafficking

Authors: *B. D. RICHARDSON[1], E. CARTIER[1], J. SWANT[1], K. SAHA[1], D. ANGOLI[1], M.-F. ZOU[2], A. H. NEWMAN[2], H. KHOSHBOUEI[1];
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Abstract: The dopaminergic system is essential for the function of the brain's reward, motor coordination, attention and cognitive processes whereas aberrations in dopamine (DA) neurotransmission contributes to neuropsychiatric disorders; including addiction, attention deficit/hyperactivity disorder (ADHD), Schizophrenia, and Parkinson's. DA neurotransmission timing and magnitude is dependent on how DAT regulates extracellular DA levels, but membrane surface expression of DAT is plastic. Regulation of DAT trafficking to and from the cell surface is sensitive to changes in multiple protein-protein interactions and substrate binding. However, many of the substrates and DA-related disease states linked to altered DAT trafficking also effect neural excitability. Since some cell signaling mechanisms known to alter DAT trafficking are also sensitive to changes in membrane conductance or excitability (e.g. CaMKII) themselves, their voltage-dependent activation/inhibition may change the cytosolic/membrane distribution of proteins like DAT.
Data in heterologous expression systems suggest that change in cellular resting membrane potential (RMP) is a regulator of DAT trafficking to and from the cell membrane. Here, we examine the regulation of DAT trafficking by a novel factor: membrane voltage/resting membrane potential (RMP). Using Total Internal Reflection Fluorescence (TIRF), confocal microscopy, whole-cell patch-clamp and cell surface biotinylation, we examined the effect of changes in the membrane voltage on the density of cell surface YFP-DAT and internalization of DAT in CHO and HEK293 cells. It was found that depolarization of the cell's RMP reduces membrane surface TIRF YFP-DAT signal and increases DAT and DAT-JHC1-064 complex

internalization. Conversely, membrane potential hyperpolarization increases membrane surface TIRF YFP-DAT density. These voltage-dependent changes in membrane surface DAT were also sensitive to CaMKII inhibition. Results indicate the sensitivity of protein trafficking to cellular electrophysiological state; providing a new mechanism dynamically regulating protein function through changes in cell surface density. Ongoing experiments using specific tools (e.g. optogenetics) to control the neuronal RMP in DAT overexpressing CHO and HEK cell lines and DAergic neurons will further determine the impact of membrane potential state on DAT density and DA uptake capacity.

Disclosures: B.D. Richardson: None. **E. Cartier:** None. **J. Swant:** None. **K. Saha:** None. **D. Angoli:** None. **M. Zou:** None. **A.H. Newman:** None. **H. Khoshbouei:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 228.18/E39

Topic: B.05. Transporters

Title: Investigating microdomain localization of the dopamine transporter using super-resolution microscopy

Authors: T. RAHBEK-CLEMMENSEN[1,2], J. ERIKSEN[1,2], S. ERLENDSSON[1,2], *T. N. JORGENSEN[1,2], U. GETHER[1,2];
[1]Dept. Neurosci. and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark; [2]The Lundbeck Fndn. Ctr. for Biomembranes in Nanomedicine, Copenhagen, Denmark

Abstract: The dopamine system is highly important for cognition, motivation, and motor functions. Dysfunctions of dopaminergic neurotransmission is coupled to neuropsychiatric disorders such as Parkinson's disease, schizophrenia, attention deficit/hyperactive disorder, and drug addiction. The dopamine transporter (DAT) is present on the presynaptic membrane of the dopaminergic neuron and mediates reuptake of released dopamine. Furthermore, DAT is a major target for psychostimulants such as cocaine and amphetamine. By conventional confocal and widefield imaging we have previously shown a homogenous distribution of DAT at the plasma membrane of cultured dopaminergic neurons [1]. Based on work with transiently transfected heterologous cells, others and we have suggested a microdomain localization of DAT through co-localization with cholera toxin beta; a known marker for lipid rafts [2, 3]. These imaging studies, however, are all limited by the physical barriers of conventional light microscopy, reaching a spatial resolution of 250 nm and above in the XY plane and 500 nm in the Z plane.

Here, we use 2 dimensional stochastic optimization reconstruction microscopy (STORM), to reach sub-diffraction limit resolution (17 nm) and show that DAT is distributed into membrane microdomains in transiently transfected CAD cells as well as primary cultures of dopaminergic neurons from the ventral tegmental area. We verified a segregated membrane distribution of mature DAT through continuous sucrose gradients from lysates of transiently transfected CAD cells as well as striatal slices. Furthermore, cholesterol depletion using methyl-beta-cyclodextrin show that for transiently transfected CAD cells this segregation is cholesterol dependent. Similar cholesterol depletion in dopaminergic neurons indicates that the microdomain clustering of DAT observed by STORM is cholesterol dependent. Finally, by combining total internal reflection microscopy with STORM and a fluorophore-conjugated monoclonal, primary antibody-only setup we will investigate the clustering nature of DAT at the plasma membrane of CAD cells.

References:

[1] J. Eriksen et al. Visualization of dopamine transporter trafficking in live neurons by use of fluorescent cocaine analogs. J. Neurosci. 29 (21), 2009.

[2] E. Adkins et al., Membrane Mobility and Microdomain Association of the Dopamine Transporter Studied with Fluorescence Correlation Spectroscopy and Fluorescence Recovery after Photobleaching. Biochemistry, 46, 10484, 2007. [3] M.L. Cremona et al., Flotillin-1 is essential for PKC-triggered endocytosis and membrane microdomain localization of DAT. Nat. Neurosci., 14 (469), 2011.

Disclosures: T. Rahbek-Clemmensen: None. **J. Eriksen:** None. **S. Erlendsson:** None. **T.N. Jorgensen:** None. **U. gether:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Program#/Poster#: 228.19/E40

Topic: B.05. Transporters

Support: NIH Grant R01 DA019676

Title: Protomers in a dopamine transporter oligomer can influence each other

Authors: T. ANTONIO[1], S.-Y. CHENG[3], J. ZHEN[1], S. ALI[2], K. T. JONES[1], *M. E. REITH[1,4]; [1]Psychiatry, [2]Physiol. and Neurosci. Mol. Neurobiol. Progr., New York Univ. Schl Med., NEW YORK, NY; [3]Sci., John Jay Col. of Criminal Justice, New York, NY; [4]Biochem. and Mol. Pharmacol., New York Univ. Sch. Med., New York, NY

Abstract: Previous studies point to quaternary assembly of dopamine transporters (DATs) in oligomers. However, the functional role of DAT oligomers is not clear. As a first step in establishing whether protomers in an oligomeric DAT assembly can function independently, we co-transfected HEK 293 cells with DAT constructs possessing differential binding affinity for the phenyltropane analog of cocaine, [^{3}H]CFT. The goal was to determine whether the binding properties in co-transfected cells (where oligomers containing the two constructs can occur) differ from those in preparations obtained by simply mixing cells that had been separately transfected (where such oligomers cannot occur). For example, single transfection with WT DAT gave a high-affinity K_d of 16 $\pm$ 3 nM, whereas single transfection with D436N DAT resulted in a low-affinity K_d of 129 $\pm$ 16 nM. Co-transfection of these two constructs yielded a K_d of 103 $\pm$ 5 nM, which differed ($P<0.005$) from the higher-affinity value of K_d of 25 $\pm$ 2 nM to be predicted for non-interacting protomers. The latter was calculated from simulating an analysis of results obtained in silico by mixing the separate binding components of high and low affinities incorporating classical Rosenthal analysis. This method for predicting resultant K_d (and B_{max}) values was validated by mixing cell lines stably transfected with either WT or D436N, and comparing predicted with "mixed" binding values. Dominance of the K_d value of a low-affinity protomer co-present with WT was also found for D345N co-transfected with WT, suggesting interaction among the protomers. In contrast, affinity values consonant with independently functioning protomers (simply mixing the two binding components) were found upon co-transfection with the following pairs of constructs with differential K_d: W84L-WT, and W84L-D345N. The results obtained so far indicate that, under some, but not all, circumstances one protomer can influence the properties of another protomer. It will be important to establish how protomers with sufficiently different DA *transport* properties influence each other.

Disclosures: T. Antonio: None. **M.E. Reith:** None. **J. Zhen:** None. **S. Ali:** None. **K.T. Jones:** None. **S. Cheng:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: NIH grant DA032857

Title: L-DOPA induces non-vesicular dopamine release

Authors: *D. XU[1], W.-X. SHI[2];
[1]Dept. of Pharmaceut. Sci., Loma Linda Univ. Sch. of Pharm., Loma Linda, CA; [2]Depts of Pharmaceut. and Basic Sic., Loma Linda Univ. Schs of Pharm. and Med., Loma Linda, CA

Abstract: L-DOPA is a precursor of dopamine (DA) and the gold-standard treatment of Parkinson's disease. L-DOPA increases DA release in both the terminal regions and somatodendritic area of DA cells in the substantia nigra (SN). SN DA release regulates both the activity of DA neurons and GABA release from striatonigral terminals. In this study, we tested whether L-DOPA still induces DA release in the SN after vesicular release is blocked by a combined inhibition of the vesicular monoamine transporter (VMAT), Ca^{2+} influx, and TTX-sensitive Na^{+} channels. We indirectly measured DA release by measuring L-DOPA-induced membrane hyperpolarization of DA neurons using whole-cell recording in rat brain slices. Under control conditions, L-DOPA-induced hyperpolarization was completely reversed by the D2 receptor antagonist raclopride and enhanced by the DA transporter (DAT) inhibitor cocaine, confirming that it is mediated by DA release and subsequent activation of DA autoreceptors. To block vesicular release, slices were first incubated with the VMAT blocker reserpine (2 μM) for at least 1 hour and then perfused with a Ca^{2+}-free medium containing TTX (1 μM) in addition to reserpine. Under such conditions, L-DOPA (100 μM) still induced a hyperpolarization of DA cells, though the amplitude (4.61 ± 0.56 mV, n=7) was significantly reduced compared to controls (7.94 ± 0.42 mV, n=6, $p<0.05$, ANCOVA). Additionally, the time between 20% and 80% of the maximal response was prolonged from 1.02 ± 0.25 min in controls to 2.57 ± 0.78 ($p=0.05$) after blockade of vesicular release, suggesting a decrease in not only the amount, but also the speed of DA release. The observation that the hyperpolarization induced by L-DOPA in the presence of blockade of vesicular release was still enhanced by cocaine (10 μM, 11.70 ± 1.61 mV, n=8, $p<0.01$, ANCOVA) and reversed by raclopride confirms that the effect is due to DA release induced by L-DOPA. Based on these results, we conclude that a large portion of DA release induced by L-DOPA is not mediated by Ca^{2+}-dependent exocytosis.
While the exact mechanism underlying the observed non-vesicular DA release remains to be determined, it is possible that L-DOPA, by increasing the DA concentration inside DA neurons, induces a reverse transport of DA through the DAT. If true, our results with cocaine would suggest that the DAT-mediated reverse transport is not blocked by cocaine or is less sensitive to cocaine than the DAT-mediated reuptake.

Disclosures: D. Xu: None. **W. Shi:** None.

Poster

228. **Dopamine Transporters: Modulation and Disease**

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Topic: B.05. Transporters

Support: Danish Medical Research Council

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Lundbeck Foundation

Title: Slc6a3-GFP: A novel transgenic mouse strain to obtain high purity isolation of dopaminergic neurons

Authors: *M. RICKHAG[1], S. STILLING[1], J. ERIKSEN[1], G. SØRENSEN[1], T. RAHBEK-CLEMMENSEN[1], J. PRAVSGAARD CHRISTENSEN[2], U. GETHER[1];
[1]Molecular. Neuropharm Lab, Dept. of Neurosci & Pharmacol, Univ. of Copenhag, Copenhagen, Denmark; [2]Dept. of Intl. Health, Immunol. and Microbiology, Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Dopamine (DA) plays a fundamental role in the central nervous system as a modulatory neurotransmitter, controlling different brain functions including locomotor activity, reward mechanisms, cognition and neuroendocrine functions. Dopaminergic neurons are primarily localized in substantia nigra (SN) and ventral tegmental area (VTA) of the midbrain and project to areas in the basal ganglia, prefrontal cortex and the limbic system. However, only a fraction of the cells in the VTA and SN are dopaminergic, therefore, markers permitting specific visualization and identification of dopaminergic neurons in this area are highly desirable. We have obtained a BAC transgenic mouse strain from GENSAT, which expresses enhanced green fluorescent protein (GFP) under control of the dopamine transporter (DAT) promoter (Slc6a3-GFP). The presynaptic DAT is responsible for sequestering released dopamine from the synaptic cleft and is selectively expressed in DA neurons. Immunohistochemical analysis showed robust endogenous GFP expression in both ventral midbrain and striatal terminals that co-localized with dopaminergic markers. Antibody labelling of GFP also showed extensive overlay with DAT in both cell soma and extensions of DA neurons in midbrain validating that GFP is a reliable marker of dopaminergic neurons in Slc6a3-GFP mice. Furthermore, we did not observe any functional or behavioural abnormalities in these mice compared to non-transgenic wildtype mice when assessing striatal dopamine uptake, basal locomotion and psychostimulant-induced hyperactivity. Using fluorescence activated cell sorting, we demonstrate that a pure dopaminergic cell population can be obtained from Slc6a3-GFP mice as evidenced by enrichment of gene transcripts highly expressed in dopaminergic cells. Furthermore, the GFP expression in *Slc6a3*-GFP mice will in combination with fluorescent cocaine analogues allow direct visualization of DAT trafficking in live striatal slices. We believe that Slc6a3-GFP mice represent a novel tool for high-purity isolation of dopaminergic neurons

that can be used in transcriptomic and epigenetic analyses as well as for high-yield dopaminergic primary cultures.

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Poster

228. **Dopamine Transporters: Modulation and Disease**

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NIH Grant MH095044

Vanderbilt Brain Institute Scholar's Award

Title: Dopamine signaling in *C. elegans* is regulated presynaptically by a highly conserved ortholog of the atypical MAP Kinase ERK7/8

Authors: *D. BERMINGHAM[1], J. A. HARDAWAY[1], S. M. WHITAKER[1], S. L. HARDIE[1], L. CARVELLI[2], R. D. BLAKELY[1];
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Abstract: The neurotransmitter dopamine (DA) acts across phylogeny to modulate fundamental aspects of physiology and behavior, including movement, appetite, reward and attention. The model system *Caenorhabditis elegans* is a powerful platform for the discovery and manipulation of genes controlling synaptic function, including genes that control DA production, secretion, inactivation and response. We have observed a phenotype that occurs in late larval animals where loss of the DA transporter, DAT-1, results in animals that paralyze over a course of 10 minutes when placed in water, whereas wild-type animals swim at a relatively constant rate for up to an hour. "Swimming-induced paralysis", or Swip, can be reversed pharmacologically by treatment with reserpine, which prevents vesicular DA packaging and release, as well as by genetic ablation of the DA biosynthetic enzyme tyrosine hydroxylase (*cat-2*) or the D2-like DA receptor *dop-3*. Using the Swip phenotype, we have performed a forward genetics screen to identify mutants that exhibit *dat-1* like paralysis that can be rescued by reserpine treatment as well as by mutation of genes supporting DA synthesis and response. One such mutant, *vt32*, was

localized by SNP mapping and whole genome sequencing to an uncharacterized gene, here referred to as *swip-13*. We find that *swip-13* mutations result in significantly reduced sensitivity to the neurotoxic *dat-1* substrate 6-OHDA, supporting a role for *swip-13* in sustaining DAT-1 protein expression, surface trafficking and/or activity. Consistent with these findings, transport studies in primary *C. elegans* cultures reveals a significant reduction in DA uptake. We observe that *swip-13* mutants also possess reduced DA levels, suggesting that they may either fail to recycle DA as seen in DAT knockout mice, or that the mutation has more pleiotropic actions on DA synthesis, packaging or release. Importantly, DA neuron-specific, transgenic expression of the wild-type *swip-13* gene restores normal swimming behavior of *swip-13* mutants, establishing expression by DA neurons as the key site of SWIP-13 expression to modulate DA signaling . Fluorescently-tagged, functional *swip-13* protein localizes to DA terminals, consistent with a presynaptic role for SWIP-13. SWIP-13 protein is highly conserved, likely representing the nematode homolog of the atypical MAP kinase ERK7/8. Further efforts to uncover the mechanisms by which SWIP-13 and ERK7/8 modulate DA signaling may provide novel insights into disorders associated with perturbed DA signaling and their treatment.

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Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Program#/Poster#: 229.01/E44

Topic: B.07. Synaptic Transmission

Support: NHMRC ID#569680

Title: Loss of α4-laminin results in aberrant neurotransmission in multiple skeletal muscle fiber types

Authors: *K. K. CHAND[1], K. LEE[1], P. G. NOAKES[1,2], N. A. LAVIDIS[1];
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Abstract: Synaptic basal lamina such as laminin-9 (α4β2γ1), have been shown to play an integral role in the organization of the neuromuscular junction (NMJ). Formation of active zones and junctional folds occur normally in α4-laminin knockout mice (α4 $^{-/-}$) as in wild-type (WT) mice. However, the precise apposition of active zones and postsynaptic folds fails to develop at α4 $^{-/-}$ NMJs. The α4-laminin chain, is found in highest concentration between the postsynaptic

folds rather than in the folds themselves, suggesting it plays an instructive role in the placement of postsynaptic specializations such as acetylcholine receptors (AChRs). Here, we compare neurotransmission at postnatal day 18 (P18) and 60 (P60) in wild type and $\alpha4^{-/-}$ NMJs. We functionally examined the consequence of α4-laminin knockout in muscles composed of predominantly three fiber types; type I, soleus; type IIB, extensor digitorum longus (EDL); and mixed type I and type IIA, diaphragm, utilising intracellular electrophysiological recordings of end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs). Mice were bred and maintained on a C57BL/6-129SvJ genetic background. Soleus, EDL and diaphragm along with associated innervating nerves were dissected from WT (n=6) and $\alpha4^{-/-}$ (n=6) mice at both P18 and P60. Immunofluorescence staining was conducted to investigate the presence of presynaptic active zone markers in relation to postsynaptic AChRs. Our results demonstrate clear aberrations in neurotransmission at $\alpha4^{-/-}$ NMJs. At P18, both MEPP and EPP amplitude increased significantly in $\alpha4^{-/-}$ NMJs compared to WT ($p<0.05$) in all fiber types. Analysis of spontaneous release displayed a two-fold increase in rise time ($p<0.05$) and a decrease in frequency ($p<0.05$) at $\alpha4^{-/-}$ NMJs across each fiber type. P18 $\alpha4^{-/-}$ NMJs displayed an increase in the frequency of evoked stimuli that failed to illicit a response in each fiber type compared to WT. At P60 increased MEPP and EPP amplitudes were observed at $\alpha4^{-/-}$ NMJs in comparison to WT ($p<0.05$). Rise time of spontaneous release also increased for each fiber type at $\alpha4^{-/-}$ NMJs compared to WT ($p<0.05$). Immunohistochemical studies demonstrated normal presence of presynaptic components in relation to postsynaptic AChRs, suggesting the necessary release machinery is present at $\alpha4^{-/-}$ NMJs but may not be organized for optimal neurotransmission. Our present findings suggest a decrease in the number of active release sites at $\alpha4^{-/-}$ NMJs across each fiber type investigated. Given the large safety factor associated with synaptic transmission at the NMJ these aberrations are not sufficiently significant to compromise the survival of the animal even at P60.

Disclosures: K.K. Chand: None. **K. Lee:** None. **P.G. Noakes:** None. **N.A. Lavidis:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: NINDS INTRAMURAL PROGRAM

Title: Organization of the presynaptic filamentous matrix in glutamatergic hippocampal synapses analyzed by EM tomography

Authors: *A. A. COLE, X. CHEN, T. S. REESE;
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Abstract: Diffusion has been thought to facilitate the transfer of synaptic vesicles to the presynaptic active zone where they dock prior to fusion. However, it is difficult to see how diffusion would work in synapses like those in squid, where synaptic vesicles cluster in vesicle clouds not restrained by the membrane of the bouton. Recently, electron microscopy (EM) tomography has shown an abundance of filamentous material between vesicles (Burette et al. 2012, Fernàndez-Busnadiego et al. 2011, Siksou et al. 2007, Szule et al. 2012), confirming that vesicles may not generally be free to diffuse. Here, we use EM tomography on thin sections of freeze-substituted, dissociated hippocampal neurons (Chen et al. 2008) to study in more detail the filamentous connections between vesicles. By freezing in lieu of initial fixation, we avoid fixation induced synaptic activity and present a picture of synaptic vesicle distribution and filament connectivity that is close to a synapse at rest. We show that vesicles in the glutamatergic boutons are constrained by two distinct clustering systems. One system consists of short filaments that connect a vesicle with up to eight neighboring vesicles. These connections show little directional bias and appear to tether the vesicles into a relatively evenly spaced, symmetrical network. The other system consists of larger, more complex, branched filaments that contact up to eight vesicles. This complex-branched system includes ~80% of vesicles, which are also cohesive with the vesicles tethered by the short-filament system. In contrast to the short-filament system, the complex-branched system is notably oriented towards the active zones and therefore positioned to move vesicles in that direction in response to tensions in the filament system - for instance, as vesicles at the active zone disappear by complete exocytosis. These two cohesive systems, working together, may represent a mechanism for both maintaining a vesicle cloud and for moving them to the active zone, in glutamatergic synapses. A comparable complex system of filaments constraining vesicle mobility is present at the active zone of the neuromuscular junction (Szule et al. 2012).

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Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: Israel Science Foundation 1427/12

Title: An ATP binding site regulates synapsin IIa function

Authors: *D. GITLER, Y. SHULMAN;
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Abstract: The synapsins are neuron-specific proteins mostly known for their capability to cluster synaptic vesicles and to control the movement of vesicles between adjacent en-passant synapses. All members of the synapsin family bind ATP at a well-conserved site in their central "C" domain. ATP binding in different synapsin isoforms is differentially regulated by calcium ions, suggesting a physiological function. However, the significance of ATP binding has not been investigated in the context of neurotransmission. We studied the effect of ATP binding on the clustering of vesicles and on their mobilization by reintroducing wildtype and mutant synapsin IIa into cultured mouse neurons lacking all synapsins. We used mutations that render synapsin IIa incapable of binding ATP (K270Q) or which alter its calcium-dependence (K374E). Using electrophysiological recordings from hippocampal autaptic neurons we found that both synIIa-K270Q and synIIa-K374E are defective in rescuing synaptic depression. In contrast, quantification of synaptobrevin 2 immunofluorescence revealed that the K270Q mutation significantly enhanced vesicle clustering, while the K374E mutation had no effect. These observations suggest that interfering with ATP binding by synapsin IIa results in hoarding of vesicles in a manner that is under-responsive to activity patterns. Consequently, we hypothesize that under normal resting conditions ATP-binding by synapsin IIa plays a significant role in organizing the vesicles into clusters close to the synaptic active zone. In addition, calcium-dependent regulation of ATP binding during intense neuronal activity may couple activity to vesicle recruitment and mobilization.

Disclosures: D. Gitler: None. **Y. Shulman:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 229.04/E47

Topic: B.07. Synaptic Transmission

Support: R01 MH077303

Title: Novel ligands of the synaptic organizer neurexin 1alpha

Authors: *G. RUDENKO[1], Y. WANG[1], L. ZHANG[2], Z. LU[2], F. CHEN[1], A. M. CRAIG[3], G. REN[2];

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Abstract: Alpha-neurexins are essential synaptic adhesion molecules implicated in neuropsychiatric diseases such as autism spectrum disorder and schizophrenia. Neurexins play a role in synapse maturation and synaptic transmission. In mammals, thousands of neurexin splice forms are generated. Alternative splicing to neurexin mRNA transcripts typically results in small stretches of amino acids being inserted at evolutionarily conserved regions in the protein; the presence or absence of specific splice inserts is known to regulate binding of certain protein partners. The extracellular domains of alpha-neurexins interact with many different endogenous proteins in the synaptic cleft, including neuroligins, LRRTM family members, alpha-dystroglycan, latrophilin, cerebellins, neurexophilin and the GABAA-receptor. Unlike the much smaller beta-neurexins that contain a single LNS domain in their extracellular domain, the extracellular domains of alpha-neurexins are comprised of 9 modules, i.e. 6 LNS domains interspersed by 3 EGF-like repeats (over 1300 residues). Our structure of the neurexin 1alpha extracellular domain determined to 2.65 Å reveals that putative protein partner binding sites, also known as 'hyper-variable surfaces', map to one side of the L-shaped extracellular domain. Mapping the sequence conservation between alpha-neurexins onto the surface of the neurexin 1alpha ectodomain reveals a characteristic distribution and suggests that novel partners are likely. We describe our approaches to identify novel partners for neurexin 1alpha and the identification of calsyntenin-3 as a novel alpha-neurexin specific protein partner. Our data provide insight into how alpha-neurexins can use the architecture of their extracellular domains to recruit multiple different synaptic protein partners and organize them into large macromolecular complexes in the synaptic cleft promoting synapse function.

Disclosures: G. Rudenko: None. **Y. Wang:** None. **L. Zhang:** None. **Z. Lu:** None. **F. Chen:** None. **A.M. Craig:** None. **G. Ren:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: UK Medical Research Council

European Union Seventh Framework Program EUROSPIN project

MEXT/JSPS KAKENHI Grant (24700404)

Title: Trans-synaptic regulation of presynaptic organization and function by postsynaptic N-Cadherin

Authors: *T. SHINOE, Y. GODA;
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Abstract: N-cadherin is the most widely expressed classic cadherin family member in the CNS. Localized to the pre- and the postsynaptic sites, homophilic interaction of N-cadherins across the synaptic cleft plays important roles in the brain. Previous studies have shown that interfering with N-cadherin-dependent adhesion results in fewer synapses, more spines with immature properties, and attenuation of long-term synaptic plasticity. Compromising N-cadherin activity selectively in postsynaptic neurons decreases the expression level of presynaptic proteins, the number of synaptic vesicles, and the basal release probability. Moreover, this trans-synaptic impairment is accompanied by delayed vesicle endocytosis. In contrast, presynaptic disruption of N-cadherin activity does not cause similar presynaptic impairments. These results strongly suggest that postsynaptic N-cadherin activity can regulate presynaptic function without cooperating with presynaptic N-cadherin. We sought to clarify this novel trans-synaptic regulatory mechanism in cultured hippocampal neurons using optical and electrophysiological approaches. Total vesicle pool size, recycling pool size, and exo/endocytic properties were investigated in the VGLUT1-pHluorin expressing-presynaptic terminals forming synapses with the postsynaptic neuron overexpressing either mCherry-tagged wild-type N-cadherin, a dominant-negative N-cadherin, or a mCherry control. We also addressed the role of GluA2 extracellular domain that directly binds to N-cadherin extracellular region. Overexpressing GluA2 extracellular domain-deletion mutant, GluA2ΔN showed the same effects as impairing postsynaptic N-cadherin activity where it significantly decreased miniature EPSC frequency and the number of presynaptic terminals received by the GluA2ΔN-expressing neurons and their expression level of VGLUT1. Our findings suggest that GluA2 extracellular region is part of a novel regulatory mechanism that could cooperate with postsynaptic N-cadherin to control presynaptic organization and function in *trans*.

Disclosures: T. Shinoe: None. **Y. Goda:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Program#/Poster#: 229.06/E49

Topic: B.07. Synaptic Transmission

Support: Deutsche Forschungsgemeinschaft

Title: Transsynaptic modulation of the synaptic vesicle cycle by synaptic adhesion molecules

Authors: B. VAN STEGEN, *K. GOTTMANN;
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Abstract: Synaptic adhesion molecules are well known to mediate transsynaptic signaling that controls the formation, maturation, and function of synapses in the brain. The adhesion molecule N-cadherin is present at excitatory glutamatergic synapses and interacts homophilically across the synaptic cleft thus forming the N-cadherin/catenin adhesion complex. In immature mouse cortical neurons, N-cadherin has been described to indirectly control presynaptic vesicle accumulation by targeting and activating postsynaptic Neuroligin1, which in turn induces presynaptic vesicle clustering via Neurexin binding. The N-cadherin and Neuroligin1 adhesion systems have further been proposed to be molecularly linked by postsynaptic scaffolding proteins.

We have now studied the functional role of N-cadherin and Neuroligin1 at mature glutamatergic synapses by postsynaptically overexpressing these adhesion molecules in long-term cultured cortical neurons. The vesicle cluster-inducing, synaptogenic activity of Neuroligin1 was absent after intrinsic synaptogenesis had ceased as demonstrated by VAMP2 immunocytochemistry. However, staining cycling vesicle clusters with FM dye revealed an enhanced vesicle cycling upon Neuroligin1 overexpression. Intriguingly, overexpression of N-cadherin also resulted in a similar enhancement of synaptic vesicle cycling. This suggests that the molecular cooperation of the N-cadherin and Neuroligin1 adhesion systems may be also of functional importance at mature glutamatergic synapses.

To further study the role of synaptic adhesion molecules at mature synapses, we have performed fluorescence imaging of exo- and endocytosis of synaptic vesicles using Synaptophysin-pHluorin (SypHy). This high-resolution technique will enable us to study the functional role of N-cadherin, Neuroligin1, and their cooperation in the retrograde modulation of the synaptic vesicle cycle.

Disclosures: B. van Stegen: None. **K. Gottmann:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Program#/Poster#: 229.07/E50

Topic: B.07. Synaptic Transmission

Support: WCI 2009-003 NRF

Title: Analysis of synapsin III function at the squid giant synapse

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Abstract: Among the 3 synapsin genes, synapsin III is unique because of its J domain. This domain contains a MAPK phosphorylation site, S470, that is mutated in some schizophrenia patients (Biol. Psych. 55: 118). To examine the function of the J domain, we injected J domain peptides into squid giant presynaptic terminals. One J domain peptide (J2) reversibly inhibited synaptic transmission. A scrambled version of this peptide did not cause any inhibition, showing that the effect of the J2 peptide is sequence-specific. Estimation of intraterminal peptide concentration indicated that half-maximal inhibition of synaptic transmission was achieved at a J2 concentration of 0.8 mM. To determine the site of inhibition, we examined synaptic depression kinetics during peptide injection. J2 accelerated the time constant of depression, indicating that the peptide inhibits synaptic transmission by disrupting the synaptic vesicle reserve pool. Thus, like the C and E domains of synapsins, the J domain is involved in maintaining vesicles in the reserve pool. To examine the function of MAPK phosphorylation, the MAPK phosphorylation site within J2 was mutated by replacing a serine residue with aspartate (J2D). This pseudophosphorylated J2D peptide was capable of inhibiting synaptic transmission, while a non-phosphorylatable version (J2N) did not. The half-maximal concentration of J2D required to inhibit synaptic transmission (0.4 mM) was lower than that required for J2, suggesting that J2 peptide must be phosphorylated before it can block synaptic transmission. Consistent with this hypothesis, the time required for J2 to inhibit synaptic transmission was significantly longer than that required for J2D to act. Differences in latency indicate that J2 requires 7 min to be phosphorylated within the presynaptic terminal. In summary, our results indicate that interactions mediated by the J domain of synapsin IIIa can regulate reserve pool size and that this function is controlled by MAPK phosphorylation.

Disclosures: S. Song: None. **G.J. Augustine:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: Hertie Foundation, CIN (Exc. 307)

NENS fellowship to D.T. Asede

Title: Amygdala intercalated cells provide presynaptically - modulated sensory feed-forward and feedback inhibition to the basolateral amygdala

Authors: *D. T. ASEDE[1,2], D. BOSCH[1], F. FERRAGUTI[3], I. EHRLICH[1];
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Abstract: During classical fear conditioning, sensory information is relayed from specific thalamic and cortical regions and converges in the lateral amygdala (LA). Properties and plasticity of these inputs onto glutamatergic neurons is well studied, but recruitment of inhibitory neurons is still poorly understood. GABAergic intercalated neurons (ITCs) surrounding the BLA in distinct clusters have recently been shown to be activated differentially during high and low fear states. Particularly, cells in the medial paracapsular cluster (mpITCs) appear to have additional outputs apart from the canonical view that they inhibit the central amygdala. Here, we address two questions: (1) Are mpITCs innervated by sensory inputs and what are their properties? (2) Are there novel targets of mpITCs within the amygdala?
Using tracing, electrical and optogenetic stimulation, and patch clamp recordings in brain slices of adult mice, we show that mpITCs receive monosynaptic sensory inputs from the same thalamic and cortical regions that innervate the LA. These inputs are glutamatergic, and mediated by AMPA- and NMDA-receptors. Both thalamic and cortical inputs express functional GABA(B) receptors, and activation leads to a presynaptic depression. Strong activation of the mpITC network by priming either thalamic or cortical afferents with high frequency stimulation, resulted in presynaptic inhibition of the other pathway, which was blocked by a GABA(B)-receptor antagonist. This suggests that sensory inputs to mpITC cells are under presynaptic modulatory control via GABA(B) heteroreceptors that can be physiologically activated by recruitment of the mpITC intrinsic GABAergic network.
To identify outputs of recorded mpITCs, we filled cells and reconstructed their axonal arbors. We identify mpITCs with axonal patterns in accordance with three published cell-types and a population with significant axonal arbors in the BLA that establishes inhibitory synaptic contacts with dendrites of BLA neurons. In keeping, retrograde tracing from the BLA labels a subpopulation of mpITCs, indicating that mpITCs provide sensory-driven feed-forward inhibition to the BLA. Furthermore, retrogradely labeled mpITCs receive monosynaptic excitatory inputs from optogenetically activated BLA principal cells in their target region. This suggests that mpITCs can also participate in feedback inhibitory control of the BLA.

Taken together, we describe novel sensory inputs to mpITCs and provide evidence that mpITCs are part of feed-forward and feedback inhibitory circuits controlling the BLA. These findings extend and challenge the classical view of ITC function in amygdala networks.

Disclosures: D.T. Asede: None. **D. Bosch:** None. **F. Ferraguti:** None. **I. Ehrlich:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

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Title: The conserved isoforms of Miro may have different roles for mitochondrial biology in axons of *Drosophila*

Authors: *M. BABIC[1], T. M. KUHN[2], K. E. ZINSMAIER[2];
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Abstract: Neuronal function critically depends on the prolonged presence of mitochondria in axons and dendrites. However, the molecular mechanisms underlying the specialized biology of axonal mitochondria remain poorly understood.
Previous work suggested that the mitochondrial GTPase Miro is critical for mitochondrial transport in axons and dendrites by coupling mitochondria to kinesin motors via the adaptor protein Milton (OIP98/106/TRAK1/2). In addition, Miro may play a critical role for the organization and health of mitochondria, possibly by coordinating transport with mitochondrial fusion and fission, degradation and mitochondria-ER interactions.
The *Drosophila miro* gene expresses three protein isoforms that differ in length by only 21 amino acids, termed Miro-long, -medium and -short (Miro-L, -M, -S). The precise location of this variable domain (VD) within the Miro protein sequence (~15 aa from the TM domain) is conserved from flies to humans but the length and sequence is not, not even between mouse and human. Hence, the VD has no discernible function other than possibly affecting the distance of the GTPase and EF-hand domains from the OMM, which could have functional consequences. To test whether Miro-L, -M, and -S are functionally equivalent, we expressed each isoform in *miro* null mutant *Drosophila*. Surprisingly, neuronal expression of Miro-M restored a normal viability, but expression of Miro-L or -S did not. Moreover, neuronal overexpression of Miro-L or -S (but not -M) caused premature death during development with a few escapers surviving to

adulthood that failed to inflate their wings and died prematurely within several hours. Further analyses revealed differential effects on mitochondrial transport, distribution, structure and health in larval motor axons. Most prominently, Miro-L (and to a lesser degree -S) expression induces the formation of 2 very different abnormal subpopulation of mitochondria: severely enlarged mitochondria with an extremely increased density of cristae and severely thin, highly elongated mitochondrial structures that are interconnected in a complex network. Interestingly, overexpression (OE) of Parkin or Pink 1 suppresses the effects on lethality induced by Miro-S expression but not that of Miro-L. Hence, the different phenotypes induced by Miro-L, -M, and -S and different genetic interactions support the notion that they are functionally different.

Disclosures: M. Babic: None. **T.M. Kuhn:** None. **K.E. Zinsmaier:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: GSU Brains and Behavior Seed Grand COOB

Title: Glutamatergic and GABAergic projections from the medial amygdala to the posterior bed nucleus of the stria terminalis

Authors: *A. R. BURNS[1], D. SHUKLA[2], L. A. MARTINEZ[2], B. M. COOKE[2], A. PETRULIS[2];
[2]Neurosci. Inst., [1]Georgia State Univ., Atlanta, GA

Abstract: The perception and processing of socially relevant odors is critical to the execution of reproductive behavior in rodents. The bed nucleus of the stria terminalis (BNST), in conjunction with the medial amygdala (MeA), plays an important role in the integration of hormonal and chemosensory cues. The posteromedial BNST (BNSTpm) has numerous steroid-sensitive cells and receives projections from the posterodorsal medial amygdala (MePD) whereas the posterointermediate BNST (BNSTpi) receives a large input from the chemosensory anterodorsal medial amygdala (MeAD). However, the neurotransmitter phenotype of the connections from the different subnuclei of the MeA to the BNST has yet to be characterized. Consequently, we investigated the phenotype of these projections with injections of the anterograde tracer biotinylated dextran amine (BDA) into the MeAD and MePD. Presynaptic boutons were labeled with a marker of glutamatergic terminals, vGlut2, and a marker of GABAergic terminals, GAD65. The extent of colocalization between the BDA-labeled axons and the presynaptic

markers was then quantified. The colocalization of vGlut2 with BDA in the BNST indicates that the proportion of glutamatergic terminals is independent of the injection site (MeAD or MePD) and BNST subnucleus (BNSTpi or BNSTpm). Similarly, colocalization of GAD65 with BDA in the BNST does not vary with injection site or subnuclei. Our preliminary analysis confirms that projections from the MeAD and MePD project selectively to the rostral BNSTpi and BNSTpm, respectively. In contrast, both caudal BNSTpi and BNSTpm appear to be innervated by projections from the MePD. This pattern was not seen in projections from the MeAD, where fibers were most abundant in the BNSTpi throughout the rostral-caudal axis.

Disclosures: A.R. Burns: None. **D. Shukla:** None. **L.A. Martinez:** None. **B.M. Cooke:** None. **A. Petrulis:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: NIH Grant K01DK081444

Title: Increased post-tetanic asynchronous neurotransmitter release at parasympathetic major pelvic ganglion neurons of type 2 diabetic mice may involve altered mitochondrial regulation of Ca2+ homeostasis

Authors: *J. D. TOMPKINS, R. PARSONS;
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Abstract: We previously determined that the frequency of tetanic stimulation-induced miniature excitatory postsynaptic potentials (mEPSPs) is substantially greater in parasympathetic major pelvic ganglion (MPG) neurons of type 2 diabetic mice. Recently we sought to determine, 1) if post-tetanic mEPSP activity correlates with the severity of hyperglycemia and hyperlipidemia in multiple strains of type 2 diabetic mice and 2) if the increased mEPSP activity is associated with impaired mitochondrial regulation of nerve terminal Ca2+. Post-tetanic mEPSP activity, after 5, 10 and 20 Hz (5 sec duration) of preganglionic nerve stimulation, was significantly increased in BKS-db+/+ (leptin receptor deficient) mice as early as 6 wks of age. mEPSP activity was greatest in BKS-db+/+ mice at 12 wks of age. In BL6-ob+/+ (leptin deficient), post-tetanic mEPSP frequency was greater than controls at 6 and 12 wks of age. The number of post-tetanic mEPSPs correlated with the degree of hyperglycemia and body weight in both models, with the greatest number of mEPSPs observed in severely hyperglycemic and obese BKS-db+/+ mice. To

test if the increased post-tetanic mEPSP activity was associated with [Ca2+]o, we determined the frequency of post-tetanic mEPSPs at multiple extracellular concentrations of Ca2+ . Increasing [Ca2+]o significantly increased post-tetanic mEPSP number. Mitochondrial depolarization also significantly increased post-tetanic mEPSP activity in both control and BKS-db+/+ mice. There was a smaller increment in mEPSP activity before and after mitochondrial depolarization in BKS-db+/+ mice indicating a possible involvement of mitochondrial Ca2+ uptake in the pathogenesis of nerve terminal dysfunction with diabetes. Ongoing experiments test the hypothesis that impaired intraterminal Ca2+ homeostasis with type 2 diabetes alters presynaptic regulation of neurotransmitter release.

Disclosures: J.D. Tompkins: None. **R. Parsons:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Title: Functions of nuclear distribution proteins in axonal mitochondrial transport

Authors: *Y. SHEN[1], C.-Y. SHAO[2], J. ZHU[2], Y.-J. XIE[2], T.-H. ZHOU[3];
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Abstract: Neurons critically depend on the long-distance transport of mitochondria. Motor proteins kinesin and dynein control anterograde and retrograde mitochondrial transport respectively in axons. The adaptor molecules that link them to mitochondria need to be better characterized. Nuclear distribution (Nud) family proteins LIS1, Ndel1 and NudCL are critical components of cytoplasmic dynein complex. Roles of these Nud proteins in neuronal mitochondrial transport are unknown. Here we report distinct functions of LIS1, Ndel1 and

NudCL on axonal mitochondrial transport in cultured hippocampal neurons. We found that LIS1 interacted with kinsein family protein KIF5b. Depletion of LIS1 enormously suppressed mitochondrial motility in both anterograde and retrograde directions. Inhibition of either Ndel1 or NudCL only partially reduced retrograde mitochondrial motility. However, knocking down both Ndel1 and NudCL almost blocked retrograde mitochondrial transport, suggesting these proteins may work together to regulate retrograde mitochondrial transport through linking dynein-LIS1 complex. Taken together, our results uncover novel roles of LIS1, Ndel1 and NudCL in axonal mitochondrial transport.

Disclosures: Y. Shen: None. **C. Shao:** None. **J. Zhu:** None. **Y. Xie:** None. **T. Zhou:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Title: Excitation by GABA spillover in a sound localization circuit

Authors: *C. J. WEISZ, K. KANDLER;
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Abstract: The lateral superior olive (LSO), an auditory nucleus involved in horizontal sound localization, receives tonotopically organized inhibitory glycinergic inputs from the medial nucleus of the trapezoid body (MNTB). The MNTB-LSO pathway undergoes refinement before hearing onset through synaptic strengthening and silencing. In addition to glycine, developing MNTB neurons also release glutamate and GABA, but their function is poorly understood. Here, we investigate a possible role for GABA. Whole-cell voltage-clamp recordings were performed from LSO neurons in brain slices from P3-P14 C57BL/6J mice. Electrical stimulation of MNTB axons elicited post-synaptic currents (PSC) in LSO neurons. In about half of recordings an unusual PSC occurred characterized by two distinct components following a single stimulus. We term these responses “doublets”. In doublets, the second component did not occur without the first, but the first could occur without the second. Both components of a doublet reversed at -20

mV (60 mM Cl- internal, n=4), suggesting that both components are chloride currents most likely due to inhibitory neurotransmitter release from MNTB neurons. The short latency from the first to the second component (~3 ms) is faster than MNTB neurons can release neurotransmitter at these ages (300 Hz stimulation), suggesting that the two components are elicited by different populations of axons. Our results suggest that doublets are the result of GABA spillover between nearby MNTB axons: 1) The probability of recording a doublet increased with stimulus strength (increased neurotransmitter release). 2) The second component in a doublet was blocked by the GABAAR antagonist gabazine (30 µM), without loss of the first PSC (n = 9 of 17 cells). 3) Doublet occurrence was enhanced by the GABA reuptake blocker guvacine (30 µM, 3 of 9 cells). 4) Higher frequency stimulation increased the occurrence of doublets in about 20% of cells. To test the presynaptic excitatory action of GABA on MNTB axons, current clamp recordings were performed from MNTB somata and axons were filled with dye to visualize terminals in the LSO with 2-photon imaging. Focal RuBi-GABA (100 µM) uncaging at the MNTB axon depolarized the soma up to 15 mV (>500 microns distant) indicating direct axonal excitation by GABA. We suggest that GABA spillover excitation of neighboring MNTB axons may play a role in the refinement of tonotopic projections from the MNTB to the LSO.

Disclosures: C.J. Weisz: None. **K. Kandler:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

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Title: Human α-synuclein mutants E46K and A53T cause vesicle trafficking defects at the lamprey giant synapse

Authors: *R. B. WALSH[1], D. J. BUSCH[2], P. A. OLIPHINT[2], W. S. WOODS[3], J. M. GEORGE[4], J. R. MORGAN[1];
[1]Marine Biol. Lab., Falmouth, MA; [2]Dept. of Mol. Cell and Developmental Biol., The Univ. of Texas at Austin, Austin, TX; [3]Mol. and Integrative Physiol. and Neurosci., The Univ. of Illinois, Urbana, IL; [4]Div. of Biol. and Exptl. Psychology, Sch. of Biol. and Chem. Sci., Queen Mary Univ. of London, London, United Kingdom

Abstract: Parkinson's Disease (PD) is pathologically characterized by death of dopaminergic neurons and the presence of Lewy bodies, which contain aggregates of the synaptic vesicle-

associate protein α-synuclein. Multiplication of the α-synuclein gene, as well as A53T and E46K missense mutations, has been linked to familial PD. While a great deal is known about the biochemical properties of WT synuclein and the disease-related mutants, how they affect synapse structure and function is unclear. Dynamic imaging studies have revealed that overexpression of WT α-synuclein, A53T, and E46K cause general defects in synaptic vesicle trafficking. However, the specific stages of the synaptic vesicle trafficking pathway that are affected, and the underlying mechanisms, remain unknown. To address this, we took advantage of the experimental accessibility of the giant reticulospinal axons of the lamprey (Petromyzon marinus), which enables us to acutely microinject excess α-synuclein or the mutants and examine the ultrastructural effects at synapses. We have previously shown that excess WT human α-synuclein causes defects in synaptic vesicle endocytosis at stimulated synapses, characterized by a depletion of synaptic vesicles (SV) and an expansion of the plasma membrane (PM). Excess synuclein also increases the amount of large atypical internal membrane structures with only a modest increase in clathrin-coated pits and vesicles. In this study, we similarly examined the effects of A53T and E46K on synaptic vesicle trafficking. A53T and E46K are mutations in the highly conserved, alpha-helical N-terminal domain of α-synuclein. A53T possesses an alpha helical structure that is similar to WT α-synuclein, while E46K has a disrupted helix. Accordingly, A53T induced severe defects in synaptic vesicle endocytosis that were similar to those observed for WT: a 50% decrease in SVs, >33% increase in PM evaginations, >10-fold increase in total internal membrane (TIM), and a modest increase in clathrin-coated structures. In contrast, the E46K mutant caused a marked decrease in SVs and a modest increase in the TIM. However, with E46K, the PM and number of clathrin coats did not differ significantly from controls. Taken together, these data indicate that A53T and E46K cause severe, but distinct, vesicle trafficking defects at the lamprey synapse. Through these and future experiments we aim to gain a deeper understanding of the mechanisms underlying α-synuclein's pathogenic role in PD.

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Poster

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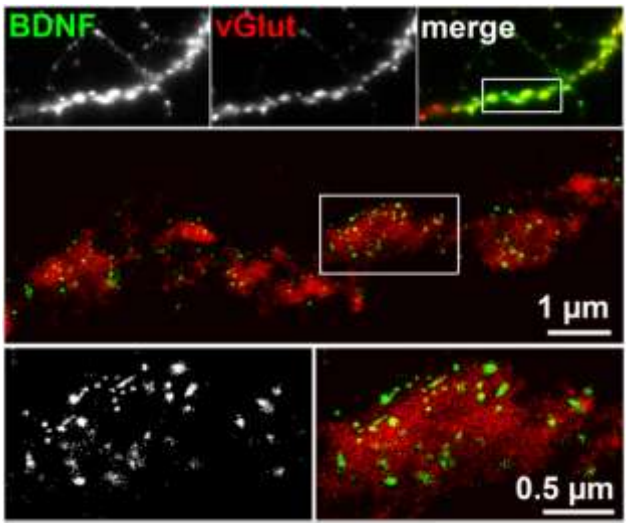
Title: Super resolution imaging reveals high amounts of BDNF in granules within presynaptic glutamatergic synapses of hippocampal neurons *In vitro*

Authors: *R. BLUM[1], T. ANDRESKA[1], S. AUFMKOLK[2], S. VAN DE LINDE[2], M. SAUER[2]; [1]Inst. for Clin. Neurobio., [2]Dept. of Biotech. and Biophysics, Univ. of Wuerzburg, Wuerzburg, Germany

Abstract: In the mammalian brain in brain-derived neurotrophic factor (BDNF) has emerged as a key factor involved in synaptic plasticity. Defects in hippocampal BDNF signaling are associated with reduced episodic memory, emotional disorders and misbalanced regulation of learned fear. Although BDNF-induced signaling cascades are beginning to be well appreciated, the spatial aspects of synaptic BDNF release are purely understood. Recent data provide evidence for an exclusive presynaptic location and anterograde secretion of BDNF in synapses of the hippocampal circuit. In contrast many studies using cultured neurons support the idea that synaptic BDNF acts retrogradely and is preferentially released from postsynaptic sites. Here we used rigorously tested anti-BDNF antibodies to achieve a dense labeling of BDNF at synaptic sites of long-term cultures of hippocampal neurons. High-resolution confocal microscopy revealed strong BDNF labels close to glutamatergic synapses. GABAergic synapses or postsynaptic neuronal structures showed only minor BDNF immunoreactivity. To ultimately resolve the BDNF distribution within the fine structure of synapses we implemented super-resolution imaging by *direct* stochastic optical reconstruction microscopy (*d*STORM) for colocalization studies with a spatial resolution of ~ 20 nm. Two-color *d*STORM images identify BDNF present mainly in small granule-like vesicles within presynaptic glutamatergic terminals (see Figure). Quantification of localization points in *d*STORM images of peripheral neurites shows that ~ 50% of all BDNF molecules in are found on the presynaptic side within glutamatergic synapses. Only about 5% of all BDNF molecules detected close to postsynaptic bars. In conclusion, super resolution imaging of endogenous BDNF reveals the principle capability of in vitro hippocampal neurons to enrich and store high amounts of BDNF within glutamatergic presynapses.



Disclosures: R. Blum: None. **T. Andreska:** None. **S. Aufmkolk:** None. **S. van de Linde:** None. **M. Sauer:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 229.16/F5

Topic: B.07. Synaptic Transmission

Support: NINDS Intramural

Title: Electron microscope tomography of synaptic cell adhesion proteins

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Abstract: Traditional electron microscopy reveals myriad organized structures, thought to represent cell adhesion proteins, which span the synaptic cleft. Several of these cell adhesion proteins-neurexin, neuroligin, cadherin, SynCAM, ephrin, and ephB-are localized to the synaptic cleft of excitatory synapses, where some of their functions have been characterized. Although electron microscopy (EM) provides the resolution to see individual molecules in the cleft, extensive overlap in a typical section makes it difficult to resolve individual proteins (Chen et al., 2008). Here we utilize EM tomography to visualize distinct individual structures in the synaptic clefts. Segmentation and rendering of these structures from 1.4 nm thick virtual sections calculated from the tomograms reveals several types of structure bridging the cleft between the

pre- and post synaptic membranes. The three most abundant types of transynaptic structure are distributed throughout the ~120 nm slice of postsynaptic density in the sample. The first type, represented by 34 copies in the sample, exhibits large, oblong pre- and postsynaptic attachments connected by a thin filament with a characteristic central nodule, while the other two types lack central nodules. The first of these, represented by 42 copies, is a filament ~4 nm in diameter within the cleft, while the other type, represented by 28 copies, is a filament ~10 nm in diameter. The two least abundant proteins spanning the cleft have distinct distributions-15 vertically oriented, doughnut-shaped complexes reside in the center of the PSD, and 9 deeply curved, filamentous structures with large presynaptic attachments and small postsynaptic attachments reside around the periphery. When the entire population of cleft structures was overlaid with renderings of presynaptic vesicles attached to the presynaptic membrane, it became apparent that transynaptic cleft structures avoid synaptic vesicle release sites. It would be premature to identify any of the cleft structures at this point, but we expect that each type corresponds to a known class of transynaptic proteins, and that eventually identifying them will provide new insights into synaptic transmission and connectivity.

Disclosures: B. High: None. **X. Chen:** None. **T. Reese:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 229.17/F6

Topic: B.07. Synaptic Transmission

Title: Using tetanus toxin (TNT) to blast the SNARE machinery

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Abstract: Tetanus toxin (TNT) causes paralysis by blocking synaptic neurotransmission through proteolysis of the v-SNARE protein synaptobrevin (VAMP2). Electrophysiological recordings at the Calyx of Held revealed that TNT leads to a decrease of evoked vesicle release, loss of the fast release component and to slowed recovery of the fast release component (Sakaba et al. 2005). These kinetic changes can be interpreted as a TNT mediated block of vesicle release sites. To examine spatial molecular reorganizations and functional alterations, TNT was conditionally expressed in *Drosophila melanogaster* larvae in a spatially and temporally restricted manner.

This allows to target a specific set of neurons and to determine the onset of TNT expression. Assessing third instar larval locomotion, we found that induction of TNT expression in motoneurons for 1200 min is sufficient to alter behaviour. Therefore this time-regime was used to induce sub-maximal TNT expression. At neuromuscular junctions amplitudes of spontaneous miniature excitatory postsynaptic currents (mEPSCs) and action potential evoked excitatory postsynaptic currents (eEPSCs) (paired-pulse stimulation, 0.2 Hz) were reduced after TNT expression in two-electrode voltage clamp (TEVC) recordings. Furthermore rise and decay times of mEPSCs were reduced and synaptic delays of eEPSCs increased by TNT.
For further analysis the organization of the filamentous active zone component Bruchpilot (Brp; Wagh et al. 2006; Kittel et al. 2006) was evaluated using a monoclonal antibody, Brp^{NC82}, directed against a C-terminal epitope of Brp (Fouquet et al. 2009). Super-resolution light microscopy of wildtype w^{1118} and TNT expressing larvae showed altered Brp-distributions in line with a reorganization of active zones (AZs). The sub-maximal blast of the SNARE machinery thus altered synaptic transmission and induced AZ growth or clustering.
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Disclosures: K. Neuser: None. **N. Ehmann:** None. **T. Martini:** None. **M. Sauer:** None. **M. Heckmann:** None. **M. Pauli:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

Location: Halls B-H

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Program#/Poster#: 229.18/F7

Topic: B.07. Synaptic Transmission

Support: HHMI

Title: Syntaxin Habc domain is required for synaptic function

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Abstract: Intracellular membrane fusion is mediated by the assembly of specific SNARE complexes. These interactions are believed to provide the driving force for bilayer fusion. The neuronal SNARE protein syntaxin (UNC-64) contains a highly conserved three-helix bundle known as the Habc domain. The Habc domain folds back to interact with its own SNARE motif, rendering syntaxin ‘’closed’’ in solution and preventing SNARE complex formation. However, it is not clear whether the Habc domain performs additional functions in synaptic transmission beside self-inhibition. In vitro studies suggest that the Habc domain is dispensable for liposome fusion. However, to date, no one has tested the role of syntaxin’s Habc domain in regulating synaptic vesicle dynamics in vivo.

To test the role of the Habc domain in vivo, we have performed rescue experiments in C. elegans. When the Habc domain of syntaxin is replaced with the homologous yeast Habc domain from Sso1, transgenes no longer rescue the unc-64 null animals. Similarly, when the SNARE motif of syntaxin is replaced with the yeast SNARE motif of Sso1, the transgene is incapable of rescuing unc-64. However, when the two chimeric proteins are expressed together, they rescue the syntaxin null. We find that this paired chimera-rescue occurs independent of the N-peptide, which has previously been shown to be important for fusion. These preliminary observations suggest that the Habc domain may facilitate fusion by a mechanism independent of self-inhibition and transport.

Since C. elegans unc-64 nulls are lethal, we are currently generating a single copy insert expressing unc-64(+) in the acetylcholine neurons to restore viability of the unc-64 null mutants. This mosaic strain will be used to express the yeast Habc domain chimera in GABA neurons to examine the role of the Habc domain in neurotransmission. Furthermore, in an effort to explore novel functions of the Habc domain, we propose to identify interacting proteins using proteomics.

Disclosures: L.A. Parra: None. **J.M. Whippen:** None. **C.Y. Dy:** None. **E. Jorgensen:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: Deutsche Forschungsgemeinschaft (SFB629, TPB11)

IZKF Münster (Mi 025/08)

NRW Research School CEDAD

Title: The α-neurexin ligand neurexophilin 3 modulate important properties of excitatory synapses

Authors: S. WANG, G. BORN, A. BLANQUÉ, A. ROHLMANN, *M. MISSLER;
Inst. of Anat. & Mol. Neurobio., Westfaelische Wilhelms Univ. of Muenster, Muenster, Germany

Abstract: The mostly presynaptic cell-adhesion molecules neurexins (Nrxns) play an essential role in synaptic transmission. However, the role of their extracellular, α-Nrxn-specific ligand neurexophilin 3 (Nxph3), a small glycoprotein that binds tightly to the second LNS domain, is unknown. Although α-Nrxn variants are expressed in virtually all excitatory and inhibitory neurons throughout the brain, Nxph3 expression is restricted to subpopulations of excitatory neurons, mostly in cerebral cortex layer 6b and in the vestibulocerebellum. To test the hypothesis that Nxph3 is a modulator of α-Nrxn function in neurotransmission, we generated a transgenic (TG) mouse model which overexpresses GFP-tagged Nxph3 under control of the Thy1.2 promoter. Morphological analysis confirmed that Nxph3-GFP in TG mice is ubiquitously expressed in many cortical neurons, and localized to the synaptic cleft, for example, of excitatory synapses in cortical layer 5 pyramidal cells which normally do not contain this molecule. Using whole-cell patch clamp recordings from these pyramidal cells in the primary somatosensory cortex, we compared synaptic transmission in Nxph3 TG and wild-type (WT) mice. While the ultrastructure of transgenic asymmetric synapses was intact, frequencies of miniature excitatory postsynaptic currents (EPSCs) were decreased in Nxph3 TG mice. Since these decreased frequencies could be rescued to normal levels by applying the $GABA_B$ receptor (R) antagonist CGP-55845, our data indicate that $GABA_BR$ are involved in the altered spontaneous neurotransmitter release. Moreover, amplitudes of electrically evoked EPSCs were also diminished in Nxph3 TG mice, which could in turn be restored to normal levels by extracellular treatment of TG neurons with the NMDAR antagonist APV. Similar rescue results could be obtained with the NMDAR antagonist MK-801 in the recording pipette, implying that postsynaptic NMDAR are involved in the altered activity-dependent neurotransmission at excitatory Nxph3 TG synapses. In support, we observed that NMDAR contributed more to EPCSs at TG synapses because NMDA/AMPA ratios were increased. As control and consistent with our immunolabeling data that showed Nxph3-GFP at excitatory synapses only, inhibitory transmission remained unchanged in TG mice. In summary, our results show that ectopic expression of the α-Nrxn-ligand Nxph3 influences basal synaptic transmission of excitatory synapses, presumably through $GABA_B$ and NMDA receptor-dependent mechanisms.

Disclosures: S. Wang: None. **G. Born:** None. **A. Blanqué:** None. **A. Rohlmann:** None. **M. Missler:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 229.20/F9

Topic: B.07. Synaptic Transmission

Support: NHMRC #ID569680

Title: α4-laminin is not involved in the developmental switch of voltage-gated calcium channel subtypes

Authors: *K. LEE[1], K. K. CHAND[1], N. A. LAVIDIS[1], P. G. NOAKES[1,2];
[1]Sch. of Biomed. Sci., The Univ. of Queensland, Brisbane, Australia; [2]Queensland Brain Inst., Brisbane, Australia

Abstract: α4-laminin has a role in aligning pre- to postsynaptic components of the neuromuscular junction (NMJ). β2-laminin that forms the laminin heterotrimer 9, with α4- and γ1-laminin is involved in organizing the presynaptic components of the developing NMJ. *In vitro* studies showed that β2-laminin binds directly to P/Q-type voltage-gated calcium channels (VGCCs) at the active zone. At postnatal day 8 (P8), neurotransmission is predominantly mediated by activation of N-type VGCCs. By postnatal day 18 (P18) there is a switch to P/Q-type VGCC dominance. As α4-laminin forms laminin-9 with β2-laminin, it is postulated that it may also be involved in this switching of VGCCs at the NMJ. In the present study we investigated the role of α4-laminin in the switch from N- to P/Q-type VGCCs at the active zones of developing P8 and matured P18 NMJs. We functionally determined the neurotransmission properties of α4-laminin-deficient mice ($\alpha4^{-/-}$) and wild types (WT) using intra- and extracellular electrophysiological recordings of evoked and spontaneous transmitter release. Mice were bred and maintained on a C57BL/6-129SvJ genetic background. Mice were sacrificed by cervical fracture with diaphragm and innervating nerve dissected free. The phrenic nerve was stimulated while recording end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) using intracellular recordings from hemi-diaphragm muscle fibres. End-plate currents (EPCs) and miniature end-plate currents (MEPCs) were recorded using focal extracellular electrodes. Immunofluorescence staining was performed using primary antibodies against N- and P/Q-type VGCCs and compared with α-bungarotoxin staining for $\alpha4^{-/-}$, β2-laminin-deficient mice and WT at P8 and P18. Electrophysiological analysis of MEPPs amplitude in $\alpha4^{-/-}$ displayed a significant increase at P8 ($n=6$, $p<0.01$) and P18 ($n=6$, $p<0.05$) compared to WT. Analysis of EPPs amplitude at P18 ($n=6$) of $\alpha4^{-/-}$ showed a significant increase ($p<0.05$) for evoked transmitter release. Intermittence in transmitter release was significantly increased in $\alpha4^{-/-}$ at P8 ($n=6$,

p<0.001) and P18 (n=6, p<0.05) compared to WT. Morphologically the distribution of N- and P/Q- type VGCCs was not changed in the $\alpha4^{-/-}$ when compared with WT at both ages examined. The present results indicate that, $\alpha4^{-/-}$ resulted in a greater intermittence which may suggest fewer active release sites. $\alpha4^{-/-}$ also showed increased MEPP and EPP amplitudes suggesting possible increased acetylcholine receptor density or decreased acetylcholinesterase activity. There was no alteration in the developmental switch from N- to P/Q-type VGCCs or the density and distribution of VGCCs at $\alpha4^{-/-}$ NMJs.

Disclosures: K. Lee: None. **K.K. Chand:** None. **N.A. Lavidis:** None. **P.G. Noakes:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 229.21/F10

Topic: B.07. Synaptic Transmission

Title: Functional role of the ATP-binding site of Synapsin I in synaptic vesicle trafficking and release dynamics

Authors: *G. LIGNANI[1], M. ORLANDO[1], L. MARAGLIANO[1], S. GIOVEDI`[3], S. GALIANI[2], A. FASSIO[3], P. BIANCHINI[2], A. DIASPRO[2], P. BALDELLI[1,3], F. BENFENATI[1,3]; [1]Neurosci. and Brain Technol., [2]Nanophysics, Inst. Italiano Di Tecnologia, GENOVA, Italy; [3]Dept. of Exptl. Med., Univ. of Genova, Genova, Italy

Abstract: The effect of energy metabolism on synaptic transmission and plasticity is a poorly explored topic. Our aim was to investigate whether presynaptic ATP concentration can be altered by intense synaptic activity and if this could affect synaptic vesicle (SV) mobilization. Synapsins (Syns) are SV−associated phosphoproteins implicated in the regulation of neurotransmitter release and synapse formation. Synapsins display a major and highly conserved ATP binding site in the central C-domain, shared by all Syn isoforms. Moreover, Syn I binds Ca2+ and Ca2+-binding regulates ATP binding. This suggests that Syn I functions, such as its capability to induce SV mobilization and trafficking, could be modulated by the Ca2+-dependent binding of ATP. To elucidate the structural basis of ATP binding to Syn I we performed Molecular Dynamics Simulations of the native protein with and without ATP and Ca2+ bound, as well as of the mutant SynI-K269Q, unable to bind ATP. To analyze the functional consequences of an alteration in ATP binding, we reintroduced either native Syn I or Syn I- K269Q mutant in Syn I knockout hippocampal neurons via lentiviral vectors. Inhibitory synaptic transmission was studied by patch-clamp coupled with genetically encoded fluorescent reporters of exocytosis

(synaptophysin-pHluorin) to evaluate SV cycling, number of active synapses, and synaptic vesicle fusion. We also used STED and electron microscopy to analyze how SVs and Syn I distribution are modified in neurons infected with native Syn I or with its mutated form both under resting conditions and upon electrical stimulation. Analysis of the ultrastructure of inhibitory synapses revealed variations in SV density and distribution in SynI-K269Q. Infection of primary neurons with Syn I-K269Q increased the strength of inhibitory transmission, altered release dynamics and impaired SV reorganization in pools. The application of sustained stimulation trains showed that the ATP mutant induced a stronger depression and a slower recovery, suggesting an altered SV supply to the readily releasable pool. Taken together these results suggest that ATP binding to Syn I plays a fundamental role in the modulation of synaptic transmission and plasticity of inhibitory synapses. Furthermore, we demonstrated that incubation of purified SVs in vitro with ATP in the absence of Ca2+ was able to increase Syn I binding to SVs and Syn I oligomerization with respect to control conditions, suggesting that Syn I might be also able to bind ATP in a Ca2+-independent manner; under these conditions SVs result more clustered, suggesting a possible direct role for ATP binding in the Syn I-induced SV clustering.

Disclosures: G. Lignani: None. **M. Orlando:** None. **L. Maragliano:** None. **S. Giovedi`:** None. **A. Fassio:** None. **P. Baldelli:** None. **F. Benfenati:** None. **S. Galiani:** None. **P. Bianchini:** None. **A. Diaspro:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Title: Mechanisms of kHz-transmission at a central synapse

Authors: *A. RITZAU-JOST[1,2], I. DELVENDAHL[1,2], A. WEYHERSMÜLLER[1,2], J. HIRRLINGER[1,3], H. SCHMIDT[1], J. EILERS[1], S. HALLERMANN[1,2];
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Abstract: The rate at which signals can be transmitted between single neurons limits the speed of information processing in the central nervous system. Cerebellar mossy fiber boutons display rates of action potentials up to 1 kHz *in vivo* [1,2]. While rapid reloading of vesicles at the release site likely sustains reliable transmission [3,4], a mechanistic analysis with presynaptic

recordings has not been performed. Here, we established paired patch-clamp recordings between cerebellar mossy fiber boutons and granule cells, presynaptic capacitance measurements, deconvolution of postsynaptic currents and quantitative presynaptic two-photon Ca^{2+}-imaging. We show that presynaptic action potentials are ultrafast (half-width 107 ± 4 μs, n = 44) and can be elicited and reliably transmitted at frequencies of at least 1 kHz. We analyze the kinetics and the Ca^{2+}-dependence of transmitter release during trains of action potentials and prolonged depolarization. The findings suggest that heterogeneous vesicle populations with Ca^{2+}-independent reloading contribute to release. Our results show that Ca^{2+}-independent reloading and heterogeneity among vesicles allow kHz transmission at a central synapse.

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Disclosures: A. Ritzau-Jost: None. **I. Delvendahl:** None. **A. Weyhersmüller:** None. **J. Hirrlinger:** None. **H. Schmidt:** None. **J. Eilers:** None. **S. Hallermann:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: RO1 HL28785

Title: Adrenergic C1 neurons drive central noradrenergic (NE) neurons via monosynaptic glutamatergic synapses

Authors: *B. B. HOLLOWAY, R. L. STORNETTA, G. BOCHORISHVILI, K. E. VIAR, P. G. GUYENET;
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Abstract: C1 neurons, located in the rostral ventrolateral medulla (RVLM), are activated by hypotension, hypoglycemia, hypoxia, infection, pain and psychological stress. C1 activation increases sympathetic tone and peripheral norepi- and epi-nephrine via direct excitation

sympathetic preganglionic neurons in the intermediolateral column. C1 neurons also target the locus coeruleus (LC) and other noradrenergic brainstem regions such as the A2 neurons located in the nucleus of the solitary tract and the A1 neurons located in the caudal VLM. In this study we examined whether the C1 cells establish synapses with these three groups of CNS noradrenergic neurons, and we test whether these synapses are excitatory. C1 neurons and projections were identified by microinjecting DIO-ChR2-eYFP(or mCherry)-AAV2 into the RVLM of dopamine-β hydroxylase-Cre (DβHCre/0) mice. We showed by immunohistochemistry that >98% of the ChR2-expressing neurons were also tyrosine-hydroxylase- (TH-)-ir and that most contained PNMT. Plentiful synaptic contacts between mCherry-ir boutons and noradrenergic neurons were identified as close appositions by light microscopy (LC, A1 A2 neurons) and ultrastructurally confirmed as synapses via immunogold-silver labeling of tyrosine-hydroxylase (TH) using electron microscopy (A1 and A2 neurons). Synaptic transmission between C1 neurons and brainstem NE neurons was studied electrophysiologically in coronal brain slices from adult mice (whole cell voltage and current clamp at 23°C). ChR2-containing C1 axons were photoactivated (1 ms, 5 mW, 473nm laser light). LC neurons were identified using landmarks and cell properties and the NE phenotype of the recorded neurons was verified posthoc by histology. A1 and A2 neurons were recorded in DβHCre/0/ROSA26-tdTomato mice after verification that tdTomato red fluorescence was specifically expressed by TH-ir neurons. Photostimulation evoked 5.5±0.4ms latency EPSCs (34.8±5.0 pA at VH= -74 mV) in 53% (42/79) of LC neurons and activated these cells from 1.9±0.4 Hz to 3.1±0.7 Hz (N= 4; current clamp). The EPSCs were eliminated by tetrodotoxin, reinstated by the K-channel blocker, 4-aminopyridine and eliminated again by adding CNQX and AP5, thus demonstrating the existence of a monosynaptic glutamatergic excitatory input from the C1 cells to LC neurons. Virtually identical results were obtained in 8 A2 neurons and 5 A1 neurons. We conclude that the C1 cells have the potential of activating the release of NE throughout the CNS via their monosynaptic excitatory input to the LC and other brainstem noradrenergic neurons.

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Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: NIMH Fellowship 1F32MH100745

Simons Foundation SFARI-07-41

Title: Synaptic analysis of neurexin 2 (nrxn2) function in conditional nrxn2 knockout mice

Authors: *L. Y. CHEN[1], T. C. SUDHOF[1,2];
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Abstract: Increasing evidence suggests that neurexins (Nrxns) are presynaptic cell adhesion molecules that are critical for maintaining synaptic function. NRXN genes have been linked toautism spectrum disorders (ASD) and schizophrenia. In particular, recent studies identified patients with ASDs that have a truncating mutation of NRXN2. However, due to the lacking of fundamental understanding of Nrxn2 function, it remains unknown why Nrxn2 is associated with these disorders. Here, we made, characterized, and used conditional knockout (cKO) mice in which both the α- and β-forms of NRXN2 can be deleted by Cre recombinase. These mice allow us to investigate the synaptic function and requirements for NRXN2. The design and creation of this cKO NRXN2 mouse was confirmed by genotyping and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) to measure mRNA levels. Analysis of spontaneous miniature postsynaptic currents, evoked responses and synaptic morphology will address the precise contributions of specific NRXN isoforms to synaptic transmission and identify pathways relevant to synapse development that give rise to cognition and brain function.

Disclosures: L.Y. Chen: None. **T.C. Sudhof:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

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Topic: B.07. Synaptic Transmission

Support: BBSRC

Title: Nanoscale organization of readily-releasable vesicles in native hippocampal terminals

Authors: *F. CRAWFORD[1], V. MARRA[3], K. STARAS[2];
[1]Life Sci., [2]Univ. of Sussex, Brighton, United Kingdom; [3]Univ. of Leicester, Leicester, United Kingdom

Abstract: Small central nerve terminals rely on the efficient turnover of neurotransmitter-containing synaptic vesicles. How these recycling vesicles are organized in the terminal, what factors influence this organization, and how their structural characteristics contribute to synaptic function remain key questions in neuroscience. Here we used FM-dye labelling methods combined with ultrastructural imaging approaches to examine principles of organization of functional vesicle pools in acute rat hippocampal slices. Specifically, we stimulated Schaffer-collaterals (40 APs, 20 Hz) while FM1-43 was applied to the stratum radiatum to drive the labelling of readily-releasable vesicles in CA3-CA1 terminals. Following rapid chemical-microwave fixation, target synapses were photoconverted in the presence of diaminobenzidine, generating an electron-dense precipitate in recycling FM-dye-positive vesicles that can be readily observed in electron micrographs. We demonstrate that the readily releasable pool is 5-10% of the total vesicle pool, comparable with previous studies. We also characterize the spatial distribution of this pool, showing that it exhibits a preferential bias towards the active zone that becomes more pronounced with increasing time after the end of stimulation. The nanoscale organization of the preferentially releasable pool offers important insights into key structure-function relationships which contribute to synaptic signalling.

Disclosures: F. Crawford: None. **V. Marra:** None. **K. Staras:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 229.26/F15

Topic: B.07. Synaptic Transmission

Support: MIUR GRANT PRIN09

Title: *In vitro* monosynaptic circuits of Helix neurons as an experimental model to study synapsin knock-down

Authors: O. BRENES[1], C. N. G. GIACHELLO[3], M. GHIRARDI[2], *P. MONTAROLO[4]; [1]Dept. of Neurosci., [2]Deptament of Neurosci., Univ. of Turín, Turin, Italy; [3]Fac. of Life Sci., Univ. of Manchester, Mancheter, United Kingdom; [4]Dept Neurosci., Univ. Turin, Turin, Italy

Abstract: Synapsins (Syn) are a conserved family of presynaptic proteins, involved in the fine-tuning of synaptic transmission. In addition, they have been related to neurite outgrowth, synapse formation and plasticity, with emerging post-docking roles.
Syn mutations or deletions have been associated with epileptogenesis. Studies in mammalian models have clarified in some degree their role in this process. However, the presence of

different genes and the possible development of compensatory mechanisms hinder an accurate data interpretation. Consequently, the aim of this project is to develop an in vitro monosynaptic connection, as a reliable experimental model, in order to better investigate the effects of Syn down regulation and employ it to analyze epilepsy-related forms of Syn.
First, we cloned two antisense RNAs (asRNA) against Helix synapsin (helSyn) into a plasmid able to constitutively overexpress them. In the asRNA expressing cells we observed a time-dependent decrease of helSyn immunostaining levels, confirming protein loss. Afterward, we investigated the effect of synapsin knock-down on neuronal morphology. In this sense, asRNA expressing cells displayed a significant reduction of neurite linear outgrowth, a decrease in the branching of newly sprouting neurites and a diminution in size and number of the synaptic varicosities.
Given that Syn are also involved in the organization of synaptic vesicle pools, we measured 5-HT release. When cultured in isolation or coupled to their physiological target, helSyn knock-down cells presented an increased release, following a short tetanic stimulation, probably related to an enhanced synchronous readily realizable pool. Furthermore, in asRNA expressing cells, presynaptic long tetanic stimulations generated synaptic depression.
Since Syn deletions have been associated to epilepsy, a pathology linked to highly excitable neuronal circuits, we characterized the cellular activity. The analyses revealed no changes in resting potential or voltage threshold. However, asRNA expressing cells presented a smaller rheobase and a higher firing response to depolarizing stimulus (higher Mean Firing Frequency), suggesting a higher intrinsic excitability. In addition, when cells were exposed to a epileptogenic drug as pentylenetretazol we observed a higher percentage of cells developing abnormal discharges and longer periods of activity (especially bursting) in helSyn-depleted cells.
Taken together, these data suggest that the lack of synapsin may affect not only neurotransmitter release and neuronal connectivity, but also cell intrinsic excitability, thus leading to an altered circuitry activity.

Disclosures: O. Brenes: None. **P. Montarolo:** None. **C.N.G. Giachello:** None. **M. Ghirardi:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 229.27/F16

Topic: B.07. Synaptic Transmission

Support: AFM Grant 16026

Title: Glutamatergic and cholinergic miniature synaptic currents at the motoneuron-Renshaw cell synapse

Authors: B. LAMOTTE D'INCAMPS[1], *P. ASCHER[2];
[1]Neurophysique et Physiologie, Univ. Paris Descartes, Paris, France; [2]Univ. Paris Descartes, CNRS, Paris, France

Abstract: In newborn mice, excitation of Renshaw cells (RCs) by motoneurons (MNs) involves the co-release of glutamate and acetylcholine (ACh) and the activation of AMPA and NMDA receptors as well as of homomeric and heteromeric nicotinic receptors (nAChRs). We have previously shown that the synaptic currents mediated by these four receptors differ in both their rise times (RTs) and their decay times (DTs) (Lamotte d'Incamps and Ascher, 2008; Lamotte d'Incamps et al., 2012). We have used these differences to examine if co-release leads to "mixed " miniature synaptic currents (mEPSCs), which would indicate that ACh and glutamate are stored in the same vesicles.
The experiments were conducted on slices of neonate mice (P5-P10). The mEPSCs were recorded in the presence of TTX at -45 mV and -60 mV. We first applied 3 antagonists out of a set of 4 (APV, NBQX, MLA, DHßE) to block selectively 3 of the 4 excitatory receptors. The experiments were then repeated in the presence of only 2 of the 4 antagonists. The results, displayed on diagrams relating RT and DT of the mEPSCs, showed that:
- if the two nAChRs are available, the diagram shows a region which is not present in either of the two diagrams for single receptors, and corresponds to "mixed" mEPSCs with the very short RT characteristic of homomeric nAChRs and the long DT characteristic of heteromeric nAChRS.
- if the two glutamate receptors are available, the diagram shows a region corresponding to "mixed" mEPSCs with the short RT characteristic of AMPA GluRs and the long DT characteristic of NMDARs
- when one glutamate receptor and one nAChR are available, the diagram does not show evidence for the presence of mixed mEPSCs
We thus find evidence for co-activation by a single vesicle of either AMPA and NMDA receptors or homomeric and heteromeric nAChRs, but not of nAChRs and glutamate receptors. This situation is thus very different from that described in the tadpole spinal cord by Li et al. (2004). The simplest explanation of our results is that ACh and glutamate are stored in and released by distinct vesicles. However, one cannot exclude that the two transmitters be stored in the same vesicles and that the separation of cholinergic and glutamatergic mEPSCs be due to a postsynaptic segregation of receptors (see Dugué et al. 2005).
Supported by CNRS, Université Paris Descartes and AFM (grant 16026).

Disclosures: B. Lamotte d'Incamps: None. **P. Ascher:** None.

Poster

229. **Synaptic Transmission: Presynaptic Structure and Organization**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 229.28/F17

Topic: B.07. Synaptic Transmission

Support: Hertie Foundation and CIN (Exc. 307)

Title: Developmental regulation of GABAb-mediated modulation of sensory inputs to lateral amygdala principal neurons

Authors: D. BOSCH, *I. D. EHRLICH;
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Abstract: In adult animals, the lateral amygdala (LA) is a major site for acquisition and storage of the CS-US association during pavlovian fear leaning. Sensory thalamic and cortical pathways converge onto LA principal neurons and are under tight control of local GABAergic interneurons. Furthermore, GABAergic inhibition (mediated by GABAa and GABAb receptors) plays a critical role in synaptic plasticity and fear and extinction learning. Behavioral experiments show that fear learning in rodents first appears at the infant to juvenile transition, and differences in fear and extinction learning continue into adulthood. Here the most striking change occurs at the juvenile to adolescence transition. While adolescent mice show learning behavior similar to adults, juveniles display fear memory erasure like behavior after extinction training. Additionally, extinction only requires activity in the amygdala and is not influenced by modulators of GABAa receptor activity, which suggests developmental changes in GABAergic inhibition in the LA. However, the development of excitatory and inhibitory networks in amygdala that may underlie changes in fear and extinction learning have been largely unexplored.
Using patch clamp recordings in acute slices from infant, juvenile, adolescent and adult mice, we investigate changes in synaptic properties of excitation and feed-forward inhibition onto LA principal neurons during the critical time windows when behavioral learning changes. Our results show a significant increase in global inhibitory drive and an upregulation of sensory feed-forward inhibition onto LA principal neurons at the juvenile to adolescent transition. Furthermore, the effect of the GABAb antagonist CGP 55845 suggests an age-dependent influence of GABAergic modulation on excitatory and feed-forward inhibitory sensory inputs including effects on amplitude, paired-pulse ratios and heterosynaptic inhibition. Taken together, our data suggest a developmentally regulated change in basic synaptic properties of sensory synapses onto LA principal neurons and an increasing influence of GABAergic modulation via

GABAb receptors in mouse lateral amygdala. These changes occur at the same time when fear memory extinction switches from erasure like mechanisms to fear memory suppression.

Disclosures: D. Bosch: None. **I.D. Ehrlich:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.01/F18

Topic: B.07. Synaptic Transmission

Support: NIH MH080297-01A1

Department of Veterans Affairs

Title: Physiologically evoked DA release from LC terminals mediates long term hippocampal, CA1 synaptic enhancement

Authors: *C. C. SMITH[1], M. S. GOLDBERG[2], R. W. GREENE[1];
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Abstract: The pre-synaptic source of dopamine (DA) in the CA1 field of dorsal hippocampus was previously thought to originate from the ventral tegmental area (VTA), even though little DA transporter (DAT) positive staining is observed. We have shown that either a knockdown of TH expression in the locus coeruleus (LC) or blockade of the norepinephrine (NE) transporter (NET) in hippocampus prevents an amphetamine induced enhancement in glutamate synaptic transmission at hippocampal CA3-CA1 synapses. Importantly, this same treatment reliably induces an increase in glutamate transmission under control conditions. Whether LC terminals readily release DA under physiological conditions was unclear. Optogenetic stimulation of LC terminals from transgenic mice expressing Cre recombinase under the TH promoter (TH-Cre), induced a DA dependent increase in glutamate transmission at CA3-CA1 synapses. Moreover, this same stimulation induced a significant increase in DA release, which was blocked by the NET inhibitor nisoxetine as determined by HPLC analysis. Future work will elucidate the dynamics of DA vs. NE release from LC terminals. These findings will direct our understanding of the particular patterns of LC neuronal activity that underlie the DA mediated reward and/or salience to experience.

Disclosures: C.C. Smith: None. **M.S. Goldberg:** None. **R.W. Greene:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.02/F19

Topic: B.07. Synaptic Transmission

Support: CIHR #102572

Title: Neurotensin and its analog, D-Tyr[11]neurotensin, differentially modulate excitatory post-synaptic currents in different populations of ventral midbrain neurons

Authors: *P. BOSE[1], P.-P. ROMPRÉ[2,3], R. A. WARREN[2];
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Abstract: Neurotensin(NT) is a potent modulator of limbic neurotransmission. Ventral midbrain (VM) injection of NT or its analog, D-Tyr[11]NT1-13, for instance, produces pro-dopamine-like effects; it stimulates locomotor activity and produces lasting sensitization to the locomotor stimulant effect of systemic amphetamine. The induction of amphetamine sensitization by D-Tyr[11]NT1-13 is dependent upon activation of VM NMDA receptors, hence suggesting that the analog stimulates glutamate release in this region. This study was aimed at comparing the effects of different concentrations of NT1-13, NT8-13 and D-Tyr[11]NT1-13 on excitatory post-synaptic current (EPSC) generated in different populations of VM neurons by electrical stimulation of the afferent pathways. Horizontal VM slices (250 um) preserving afferent inputs were obtained from male Long Evans rats aged 14 to 21 days. The action potential, Ih current and membrane conductance parameters were measured after achieving whole cell configuration. Neurons were grouped according to the presence (+) or absence (-) of the Ih current. Excitatory post-synaptic current was evoked by delivering an electrical stimulation through a monopolar tungsten microelectrode placed rostral to the recording site. Bicuculline methochloride (10 uM) was included into the ACSF to block the $GABA_a$ mediated synaptic current and isolate glutamatergic EPSCs. Five minutes of baseline EPSC activity was recorded before application of NT1-13, NT8-13 and D-Tyr[11]NT1-13 (0.01uM, 0.1uM and 0.5uM) for 7 min. Only one concentration of each peptide was tested per recorded neuron. In some experiments with D-Tyr[11]NT1-13, SR142948A (0.5 uM) and SR48692 (0.5 uM) were first added to the superfusing medium before the peptide. Results show that NT1-13 and NT8-13 both produced a concentration dependent increase in EPSC amplitude in Ih(+) and Ih(-) neurons. D-Tyr[11]NT1-13 also increased EPSC amplitude in Ih(-) but decreased it in Ih(+) neurons. The NT receptor antagonists, SR142948A and SR48692, were both effective at blocking both effects of D-

Tyr[11]NT1-13, hence suggesting they are mediated by NT receptors. The higher affinity of SR48692 for NTS1 suggests that the differential effects of D-Tyr[11]NT1-13 on each population of neurons are mediated by activation this receptor. However, this can hardly account for the similar effects of NT1-13 and NT8-13, two peptides that have a higher affinity for NTS1. A possible explanation is that one of the effects of D-Tyr[11]NT1-13 is mediated by NTS2 and that at the concentration used, SR48692 also blocked this receptor sub-type; this hypothesis is currently being investigated.

Disclosures: P. Bose: None. **P. Rompré:** None. **R.A. Warren:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Support: Conicyt-PFB 12/2007 to N.C.I

Predoctoral fellowship to V.T.R

Predoctoral fellowship to F.G.S

Title: Wnt-5a acts through Gαi/0 to regulate dendritic spine formation in hippocampal neurons

Authors: *V. T. RAMIREZ, D. ORDENES, F. G. SERRANO, N. C. INESTROSA;
Pontifical Catholic Univ., Santiago, Chile

Abstract: Recently, several synaptic roles have been report for Wnt signaling pathway. Particularly, Wnt-5a activates non-canonical Wnt signaling cascade in hippocampal neurons and stimulates the development of the postsynaptic region. We have demonstrated that Wnt-5a promotes the formation of dendritic spines and increases the clustering of postsynaptic proteins as PSD-95 and modulates glutamatergic transmission, however, the molecular mechanism involved in these processes it is not fully understood. There is evidence in several models that heterotrimeric G-proteins might play a key role in the transduction of Wnt signaling, but this has not been demonstrated at least in neurons. Here, we characterized the role of G-protein in Wnt-5a signaling in a neuronal cell context and we established that the activation Gαi/0 is upstream of the Wnt-5a-induced effects in hippocampal neurons.
To address this question we first investigated the effect of pertussis toxin (PTX), an inhibitor of Gαi/0 proteins, over the Wnt-5a signaling. Neurons were stimulated with recombinant Wnt-5a

and they were pretreated or not with PTX. We observed that PTX prevents the Wnt-5a-induced activation of CaMKII and JNK. Also, pre-treatment with PTX inhibits the increase in dendritic spine number in GFP-transfected neurons and inhibits in a significantive manner the PSD-95 clustering induced by Wnt-5a.
Interesting, Mastoparan-7 (Mas-7) a specific Gαi/0 agonist, enlarges the clustering of PSD-95 and augments the number of dendritic spines. Moreover, Mas-7 activated CaMKII and JNK as Wnt-5a does. Mas-7 increases the glutamatergic transmission in mice hippocampal slices, in a similar way as Wnt-5a does. Additionally, we test if Wnt-5a promotes the activation of G protein. We used a specific antibody that recognizes the active form of Gαi/0 and we found that Wnt-5a promoted the binding of GTP to Gαi/0 at hippocampal neurons.

Disclosures: V.T. Ramirez: None. **D. Ordenes:** None. **F.G. Serrano:** None. **N.C. Inestrosa:** None.

Poster

230. **Synaptic Transmission: Modulation I**

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Topic: B.07. Synaptic Transmission

Support: NIH HD025938

P30 NS057096

Title: Modulatory role of t-cadherin in hippocampal synaptic functions

Authors: *H. BADIE-MAHDAVI[1], A. J. ROBERTS[2], B. RANSCHT[1];
[1]Neurobio., Sanford-Burnham Med. Res. Inst., San Diego, CA; [2]Neuropharm., The Scripps Res. Inst., San Diego, CA

Abstract: A growing body of evidence indicates the importance of cell adhesion molecules in regulating synapse formation, stability and function in the central nervous system (CNS). T-cadherin (Cadherin-13; Cdh13), a glycosylphosphatidylinositol-linked cadherin-type cell adhesion molecule, is prominent in diverse structures of the CNS. Mutations in the human CDH13 gene have been linked to neuropsychiatric disorders, including addiction, autism spectrum- and Attention Deficit Hyperactivity Disorder. We here report the behavioral phenotype of T-cadherin null (Tcad-KO) mice and link gene function to modulations in spine morphology and synaptic functions in the hippocampus. Tcad-KO mice show no overt phenotypes under normal housing conditions and live a normal life span. No significant

differences from wildtype (WT) controls were evident in baseline vision, hearing or locomotor activity, or spatial learning performance in the Barnes maze. However, challenge in fear conditioning tests revealed reduced freezing responses of Tcad-KO mice in both the contextual and cued paradigms that are associated with hippocampal- and amygdalar activity. Moreover, Tcad-KO mice exhibited a lower threshold for Kainic acid-induced seizure compared to WT mice consistent with modifications in hippocampal synaptic function. Polyclonal antibodies revealed strong punctate T-cadherin immunoreactivity in the hippocampus and amygdala of WT mice that was absent in the KO condition. The expression of T-cadherin in specific neuron populations in the hippocampus was confirmed by expression of Red-Fluorescent Protein (RFP) from the Rosa26 locus in Tcad-Cre mice. Compared to their WT counterparts, hippocampal slices prepared acutely from Tcad-KO mice displayed significant reductions in synaptic transmission and CA1 long-term potentiation. Spine densities of Tcad-KO CA1 hippocampal pyramidal dendrites were reduced by 28 percent, and 30 percent fewer spines showed the mature mushroom-shaped morphology in the KO than in the WT condition. Synaptic loss was confirmed by reduced expression of Synapsin I in the CA1 region of Tcad-KO mice. Our data implicate a modulatory role of T-cadherin expressed by select neuron populations in the regulation of hippocampal synaptic functions that affect behaviors encoded in components of the limbic system. **Support:** NIH HD025938 (BR); P30 NS057096 (AR).We acknowledge Ann DInh for performing some of the behavioral experiments.

Disclosures: H. Badie-Mahdavi: None. **A.J. Roberts:** None. **B. Ranscht:** None.

Poster

230. **Synaptic Transmission: Modulation I**

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Topic: B.07. Synaptic Transmission

Support: Fondecyt Grant N° 1110392

MSI Grant Nº P10/063-F

Title: An amphipatic alpha-helix in Corticotrophin Releasing Factor Binding Protein determines its sorting to the regulated secretory pathway

Authors: C. P. BASTIAS[1], E. H. BLANCO[1], *K. GYSLING[2,1];
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Abstract: Corticotrophin Releasing Factor Binding Protein (CRF-BP) is a secreted glycoprotein with high affinity for both CRF and urocortin-1 peptides. Initially, it was shown that CRF-BP binds CRF and/or urocortin-1, decreasing their activity. However, more recently, is has been proposed that CRF-BP could also facilitate CRF and/or urocortin-1 actions. Previously, we have shown that CRF-BP readily enters to the regulated secretory pathway and that its release depends on depolarizing stimuli. The purpose of the present work was to identify the sorting domain of CRF-BP necessary to enter the regulated secretory pathway. Thus, we used *in silico* prediction software of secondary structure (NPS@ Consensus Secondary Structure Prediction) to reveal CRF-BP cis-elements that could serve as the sorting signal. We found an alpha-helical structure at the N- terminal of CRF-BP. The helical wheel projection of this CRF-BP alpha-helix structure showed that it has an amphipathic configuration. Thereafter, we studied the subcellular localization and release behavior of truncated and mutated forms of CRF-BP expressed in PC12 cells. The results showed that the mutations at the level of the amphipatic alpha-helix resulted in the loss of CRF-BP sorting toward the regulated secretory pathway. Thus, our results suggest that an amphipatic alpha-helix in the N-terminal of CRF-BP is critical for its sorting to the regulated secretory pathway.

Disclosures: C.P. Bastias: None. **K. Gysling:** None. **E.H. Blanco:** None.

Poster

230. **Synaptic Transmission: Modulation I**

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Program#/Poster#: 230.06/F23

Topic: B.07. Synaptic Transmission

Support: CIHR Grant MOP259093

Title: Neurotensinergic modulation of synaptic transmission in the oval Bed Nucleus of the Stria Terminalis

Authors: *C. P. NORMANDEAU[1], C. DI PROSPERO[2], E. HAWKEN[2], E. C. DUMONT[2]; [1]Biol., [2]Queen's Univ., Kingston, ON, Canada

Abstract: Anxiety is both an adaptive and pathological behavioural state that is coordinated by different neuronal pathways. The Bed nucleus of the Stria Terminalis (BNST) seems critical in anxiety behaviours and responses to contextual stimuli. More specifically, activation of the oval subregion of the BNST (ovBNST) increases anxiety-like behaviours. However, the underlying cellular mechanism remains unknown. Neurotensin (NT) is a neuropeptide involved in anxiety and is found both in ovBNST neuronal cell bodies and synaptic terminals. Yet, the

neurophysiological role of NT in the ovBNST remains largely unclear. The objective of this project was to determine whether and how NT modulates synaptic transmission in the ovBNST using whole-cell patch clamp recordings in brain slices prepared from male Long-Evans rats. We report that exogenous application or endogenous NT release potentiated GABAergic inhibitory synaptic transmission in the ovBNST. Paired-pulse ratio analyses revealed that NT mostly acted pre-synaptically to increase the probability of GABA release. Conversely, NT had no measurable effects on glutamate release or synaptic transmission through AMPA channels. These results suggest that NT powerfully modulates inhibitory synaptic transmission in the ovBNST, a mechanism which may contribute to anxiety-like behaviours.

Disclosures: C.P. Normandeau: None. **C. Di Prospero:** None. **E. Hawken:** None. **E.C. Dumont:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

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Program#/Poster#: 230.07/F24

Topic: B.07. Synaptic Transmission

Support: NIH Grant RO1 MH83806

Title: Serotonergic excitation of amygdala projection neurons in the mouse medial prefrontal cortex

Authors: *D. AVESAR, A. T. GULLEDGE;
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Abstract: Pyramidal neurons in the rodent medial prefrontal cortex (mPFC) project to a variety of subcortical structures. We recently revealed that serotonin (5-HT) selectively excites commissural/callosal (COM) projection neurons in the mouse mPFC via 5-HT_{2A} (2A) receptor stimulation, while most other pyramidal neurons, including all corticopontine projection neurons, are functionally inhibited by 5-HT via 5-HT_{1A} (1A) receptor activation. Here we have investigated the functional impact of 5-HT on the excitability of retrograde-labeled corticoamydalar (CAm) neurons in brain slices of the mPFC from 7- to 12-week-old C57/BL6 mice. Focal application of 5-HT (100 µM; 1 second) elicited excitatory (2A-mediated) or biphasic (short 1A-dependent inhibition followed by 2A-dependent excitation) responses in CAm neurons located in both the ispilateral (n = 14) and contralateral (n = 23) hemispheres. The proportion of serotonergic responses in contralateral CAm neurons was 66% excited, 30% biphasic, and 4% having no response. In ipsilateral CAm neurons, 43% of neurons were excited

by 5-HT, and 57% exhibited biphasic responses. No CAm neurons in either hemisphere displayed a purely inhibitory response to 5-HT. Given that both COM and CAm neurons are preferentially excited by 5-HT, we tested whether these populations overlap by injecting retrograde traces of different colors into both the contralateral amygdala and contralateral mPFC. Double-labeled COM /CAm neurons (n = 11) displayed excitatory (64%) or biphasic (27%) responses, or were unresponsive (9%) to 5-HT. Finally, we found that CAm neurons had physiological properties similar, but not identical, to COM neurons. Overall, our findings reveal that CAm neurons partially overlap with COM neurons, and that both neuron types preferentially exhibit 2A-dependent excitatory or biphasic responses to 5-HT. Since CAm neurons from the mPFC participate in fear conditioning and extinction of conditioned fear, our results suggest that cortical release of 5-HT may contribute to fear responses by activation of 2A receptors.

Disclosures: D. Avesar: None. **A.T. Gulledge:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.09/F26

Topic: B.07. Synaptic Transmission

Title: Inhibitory effect of pregabalin on excitatory synaptic transmission in the spinal dorsal horn after nerve injury

Authors: R. MATSUZAWA[1], *S. YAMAGUCHI[2], T. TAKASUSUKI[2], T. OHTSUBO[3], Y. HORI[3];
[1]Anesthesiol., [3]Physiol. and Biol. Information, [2]Dokkyo Med. University, Sch. of Med., Mibu, Japan

Abstract: It is well known that pregabalin can be of value for the relief of neuropathic pain. In this study, we investigated the effects of pregabalin on synaptic transmission in the spinal cord dorsal horn after sciatic nerve ligation in mice. Tight-seal whole-cell recordings were obtained from neurons in the superficial dorsal horn of the spinal cord slices. Electrical stimulus-evoked excitatory postsynaptic currents (eEPSCs) and spontaneously occurring miniature EPSCs (mEPSCs) in the presence of tetrodotoxin were analyzed. The properties of eEPSCs and mEPSCs were observed before and after sciatic nerve ligation (Seltzer model). In sham-operated control mice, pregabalin showed a dose-dependent inhibition of amplitude of eEPSCs with EC50 of 65μM. The frequency of mEPSCs was inhibited by the bath application of pregabalin, but their amplitudes were not affected. Inhibitory effect on the frequency of mEPSCs was

significantly larger in ligated mice than in sham-operated control mice. Real-time RT-PCR analysis showed that the expression of calcium channel α2δ subunit increased in the spinal dorsal horn of sciatic nerve ligated mice, compared with sham-operated mice. Calcium channel α2δ subunit knockdown (KD) was induced by intrathecal injection of an antisense oligodeoxynucleotide. KD mice showed significant attenuation of mechanical allodynia as compared with non-sense oligodeoxynucleotide-injected control mice. In KD mice, there was no significant increase in α2δ subunit expression after nerve ligation. Our results suggest that relief of neuropathic pain by pregabalin may result from inhibition of voltage gated calcium channels containing α2δ subunits whose expression is increased after nerve injury.

Disclosures: R. Matsuzawa: None. **S. Yamaguchi:** None. **T. Takasusuki:** None. **T. Ohtsubo:** None. **Y. Hori:** None.

Poster

230. **Synaptic Transmission: Modulation I**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.10/F27

Topic: B.07. Synaptic Transmission

Title: Presynaptically mediated effects of cholecystokinin on the excitability of area postrema neurons in rat brain slices

Authors: *S. SUGETA, M. FUNAHASHI;
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Abstract: Cholecystokinin (CCK) is one of the well-known gut hormones which show anorexigenic effects via the peripheral and central receptors. Previous studies have reported that CCK inhibited feeding behavior and gastric function by acting as a paracrine modulator of vagal afferents in the periphery. CCK is also widely distributed throughout the mammalian brain and appears to function as a neurotransmitter and neuromodulator. The area postrema is one of the circumventricular organs, located on the dorsal surface of the medulla oblongata at the caudal end of the fourth ventricle. Blood vessels in the area postrema lack a blood brain barrier, offering specific central neuron components unique access to circulating substances. Immunohistochemical studies have revealed a CCK-A receptor in the area postrema. Further the presence of CCK-sensitive neurons has been demonstrated in our previous study. However, the receptive mechanism of CCK in the area postrema neurons still remains unexplained. We investigated the responses of area postrema neurons to agonists and antagonists of CCK receptors using a patch-clamp technique in rat brain slices. We found CCK-induced excitatory

responses, such as increases in the frequency of mEPSCs (miniature excitatory postsynaptic currents) and changes in the amplitude distribution of mEPSCs. These changes were found in 24 cells not displaying the hyperpolarization-activated cation current (Ih) and only 2 cells displaying Ih. An inhibitory response to CCK was never seen. CCK-induced tonic inward currents were not observed in any cells. Analysis of the amplitude of mEPSCs before and after the application of CCK indicated the responses mediated via the presynaptic receptors. The effect of CCK was abolished in the presence of CNQX (AMPA type glutamate receptor antagonist). In the presence of lorglumide(a selective CCK-A receptor antagonist)CCK-induced excitatory responses were inhibited. We conclude that CCK-induced excitation of area postrema neurons not displaying Ih is due to the facilitation of the glutamate release mediated via the presynaptic CCK-A receptors.

Disclosures: S. Sugeta: None. **M. Funahashi:** None.

Poster

230. **Synaptic Transmission: Modulation I**

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Program#/Poster#: 230.11/F28

Topic: B.07. Synaptic Transmission

Support: NSF Award IOS 1145010

Title: Functional interaction of Synapsin II and Rab3a at cultured hippocampal neurons

Authors: *H. Y. MATOS[1], R. ANDRADE[2], M. BYKHOVSKAIA[1];
[1]Neurosci., Univ. Central Del Caribe, Bayamon, Puerto Rico; [2]Dept. of Pharmacol., Wayne State University, Sch. of Med., Detroit, MI

Abstract: Synapsins are a family of phosphoproteins that have been shown to regulate the synaptic vesicle cycle and the preparatory steps for exocytosis. Synapsin gene deletion produces epileptic seizures in human patients and in animal models. Rab3a is a Synapsin binding partner, a GTP-binding protein that plays a role in the mobilization of synaptic vesicles to the active zone. Previous work has shown that Rab3a gene deletion rescues the epileptic seizures produced by Synapsin II gene deletion. We investigated excitatory and inhibitory synaptic activity in cultured dissociated hippocampal neurons of mice lacking Synapsin II (SynII(-)), Rab3a (Rab3a(-)), and both proteins (SynII(-)/Rab3a(-)) double knock out (DKO). We performed whole-cell patch clamp and paired recordings of spontaneous and evoked responses at excitatory and inhibitory synapses. Both spontaneous excitatory post-synaptic currents (sEPSCs) and miniature EPSCs (mEPSCs) frequency were increased at the SynII(-) synapses. Evoked EPSCs at SynII(-) neurons were not significantly different from those seen in wild type (WT) neurons.

In contrast, a decreased frequency was observed in both spontaneous inhibitory post-synaptic currents (sIPSCs) and miniature IPSCs (mIPSCs) at the SynII(-) neurons. These results suggest that Synapsin II deletion may disrupt the excitation/inhibition balance at hippocampal neurons. Importantly, Rab3a deletion balanced the effect of Synapsin II deletion on inhibitory transmission. More specifically, we did not observe a significant alteration in either sIPSC or mIPSC frequency at either SynII(-)/Rab3a(-) DKO or Rab3a(-) synapses compared to WT inhibitory synapses. This suggest that the deletion of Rab3a may compensate the over excitation observed at SynII(-) at inhibitory synapses.

Disclosures: H.Y. Matos: None. **R. Andrade:** None. **M. Bykhovskaia:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.12/F29

Topic: B.07. Synaptic Transmission

Support: NIH Grant MH082881

Title: Promiscuous interactions of dopamine augment GABA release within rat entorhinal cortex

Authors: *N. I. CILZ, L. KURADA, B. HU, S. LEI;
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Abstract: The entorhinal cortex (EC) acts as a gate for the majority of cortical input to the hippocampus and plays an important role in learning and memory. Dopamine (DA) is a neuromodulator known to influence learning and memory processes and the EC receives DAergic input from midbrain neurons. Whereas DA has a largely suppressive role on the excitability of the principle neurons within the EC, the relationship between DA and the GABAergic interneurons remains less clear. Using a whole-cell patch-clamp analysis, we investigated this relationship by making both postsynaptic and direct recordings from interneurons. Our results show that DA (10-100 μM) increases the frequency but not amplitude of spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs), suggesting a pre-synaptic site of action for DA. Pharmacological interventions indicate that this effect is mediated by α-1 adrenergic receptors (α1ARs) and not DA receptors. The DA-induced increase in frequency of both sIPSCs and mIPSCs requires extracellular Ca2+ influx and involves T-type Ca2+ channels. Recordings from interneurons indicate that DA increases the intrinsic excitability and depolarizes the resting membrane potential by approximately 4 mV. This depolarization is independent of extracellular Ca2+ and Na+ influx. Substitution of intracellular K+ with N-

methyl-D-Glucamine prevented DA-induced depolarization. DA-induced currents generated with a voltage ramp show a reversal potential close to the calculated K+ reversal potential and exhibit inward rectification. Depolarization was blocked by both the selective α1ARs antagonist corynanthine and blockade of inward rectifying K+ channels (Kirs) by Ba+. Taken together, our results suggest a sequential mechanism by which DA enhances GABA release. DA activates α1ARs, which initially depolarizes interneurons via inhibition of Kirs, and permits secondary activation of T-type Ca2+ channels and Ca2+ influx, ultimately increasing GABA release.

Disclosures: N.I. Cilz: None. **L. Kurada:** None. **B. Hu:** None. **S. Lei:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.13/F30

Topic: B.07. Synaptic Transmission

Support: Swedish Research Counil

Umeå university

Title: Exclusive glycine receptor-mediated inhibition to forelimb motoneurons in adult mouse spinal cord

Authors: *J. JIANG, B. ALSTERMARK;
Dept. of Integrative Med. Biology, Section of Physiol., Umeå, Sweden

Abstract: It is widely accepted that glycine and GABA mediate inhibitory synaptic transmission in the brain stem and spinal cord. The abundance of GABAergic and glycinergic terminals and postsynaptic clusters of their distinct receptors on motoneurons (MNs) suggests that both glycine and GABA exert important effects in controlling movement(1, 2). Since some of them are co-localized in the same terminals, they even can act as co-transmitters and mediate co-transmission(3). However, it also poses the question about their relative contribution to inhibition in MNs. Here, we used in vivo intracellular recordings from forelimb MNs in adult mouse to investigate the inhibitory transmission from the reticulospinal tracts, the propriospinal neurons and forelimb afferents. We found that the glycine receptors antagonist Strychnine can completely block inhibitory postsynaptic potentials (IPSPs), whereas the GABAA receptors antagonist Gabazine has no significant effect on postsynaptic potentials. Moreover, all IPSPs remaining after Gabazine disappeared after adding Strychnine. Our results show that, in the intact adult mouse, the inhibitory actions on MNs exerted by the reticulospinal tracts, the propriospinal

neurons and forelimb afferents are all exclusively glycinergic. The findings may help us understand neurodegenerative disease mechanism characterized by progressive loss of MNs, like amyotrophic lateral sclerosis (ALS) which shows abnormal synaptic inhibition resulting from dysfunction of Glycine receptors(4).

1Ornung, G. et al. Qualitative and quantitative analysis of glycine- and GABA-immunoreactive nerve terminals on motoneuron cell bodies in the cat spinal cord: a postembedding electron microscopic study. J Comp Neurol. 365, 413-426. (1996).
2Todd, A. J. et al. Colocalization of GABA, glycine, and their receptors at synapses in the rat spinal cord. J Neurosci. 16, 974-982. (1996).
3Jonas, P. et al. Corelease of two fast neurotransmitters at a central synapse. Science. 281, 419-424. (1998).
4Martin, L. J. & Chang, Q. Inhibitory synaptic regulation of motoneurons: a new target of disease mechanisms in amyotrophic lateral sclerosis. Mol Neurobiol. 45, 30-42. (2012).

Disclosures: J. Jiang: None. **B. Alstermark:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.14/F31

Topic: B.07. Synaptic Transmission

Support: GACR P303-11-0131

Title: Modulation of excitatory synaptic transmission by the somatodendritic release of glycine in the rat medial nucleus of the trapezoid body

Authors: *M. KRALIKOVA[1], R. TURECEK[2];
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Abstract: The somatodendritic release (SDR) of neurotransmitters fulfills a significant physiological role in the central nervous system. SDR can quickly deliver relatively high concentrations of neurotransmitters to extrasynaptic sites where they effectively modulate neuronal excitability. Glycine, the main inhibitory transmitter in the spinal cord and brainstem, is typically released from nerve terminals to mediate phasic synaptic communication. However, little is known about the SDR of glycine. We studied the mechanisms and relevance of the SDR of glycine in the glycinergic principal cells (PCs) forming the medial nucleus of the trapezoid body (MNTB), one of the brainstem auditory nuclei. In the MNTB, glycine is known to activate

both pre- and postsynaptic strychnine-sensitive chloride permeable receptors (GlyRs). Using patch-clamp recordings from the somata of PCs in MNTB slices, we observed tonic outwardly-rectifying and strychnine-sensitive currents. A detailed examination of the currents pharmacologically stimulated by filling the cells with a glycine-containing solution revealed their dependence on Na+ and their sensitivity to ALX1393. This indicated that glycine could be released from the PCs by a reverse action of glycine transporter 2 (GlyT2). The presence of GlyT2 proteins in MNTB principal cells was confirmed by immunohistochemistry. Our further experiments showed that SDR could be triggered by a Na+ influx during bursts of postsynaptic action potentials. In addition to postsynaptic GlyRs, extracellular glycine resulting from pharmacologically stimulated SDR also efficiently activated presynaptic GlyRs at giant nerve terminals, the calyces of Held. The activation resulted in a potentiation of the presynaptic release probability of glutamate from the calyx. Our results suggest a novel retrograde mechanism of modulation of excitatory synaptic transmission by glycine released by activity-dependent reverse uptake from postsynaptic cell compartments.

Disclosures: M. Kralikova: None. **R. Turecek:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.15/F32

Topic: B.07. Synaptic Transmission

Support: NIH Grant AG041360

Title: Rapid membrane estrogen receptor activation increases the excitatory/inhibitory ratio in rat basal forebrain slices

Authors: *J. C. DAMBORSKY, D. W. DUBOIS, A. S. FINCHER, C. ROTH, W. H. GRIFFITH;
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Abstract: Estrogen can exert rapid, non-genomic actions on cells in the brain via plasma membrane receptors, including ERα and β, and the G-protein coupled estrogen receptor (GPER). This rapid estrogenic signaling can have dramatic effects on calcium signaling and synaptic transmission, with varying effects observed in individual cell types and brain regions. Previously, our lab has shown that 17 β-estradiol (17βE) can reduce the frequency of spontaneous inhibitory synaptic transmission onto acutely dissociated basal forebrain (BF) neurons. Here, we extend these findings by looking at the effects of 17βE on excitatory and

inhibitory synaptic transmission in BF neurons in slices with more intact local circuits. We recorded spontaneous and evoked EPSCs and IPSCs from basal forebrain neurons in acute brain slices from young adult F344 female rats using whole-cell patch clamp recording techniques. We then bath applied either 17βE (100 nM), the ERβ agonist DPN (100 nM), or the GPER agonist G-1 (100 nM) to slices and recorded changes in synaptic activity. Similar to what was observed in the reduced synaptic preparation, we found that 17βE reduced sIPSC frequency in a majority of cells (6/10) with an average decrease in those cells of 23 ± 6% (n=6). Additionally, 17βE reduced the amplitude of evoked IPSCs and increased paired-pulse ratios (PPRs) in 6/10 cells, suggesting a decrease in GABA release probability. These effects may be at least partially mediated by activation of ERβ, as we found that in response to DPN, 3/5 cells showed a reduction in sIPSC frequency, and 4/5 cells had reduced evoked IPSC amplitudes or increased PPRs. Following bath application of G-1, 2/6 cells had a reduction in sIPSC frequency, and 3/6 cells showed a reduction in evoked IPSC amplitude or an increase in PPR. Interestingly, when we looked at excitatory synaptic transmission, we observed an estrogen-induced increase in synaptic transmission. Following bath application of 17βE, 8/11 cells exhibited an increase in sEPSC frequency (average increase of 47 ± 13%, n=8), 7/11 had an increase in evoked EPSC amplitude, and 7/11 had a decrease in PPR. These data from BF neurons suggest that in the presence of estrogen there is an overall increase in the excitation/inhibition ratio as a result of reduced inhibitory signaling and enhanced excitatory signaling. We observed a similar global increase in excitation in the CA1 hippocampus of adult female rats; in response to both 17β-E and G-1, we saw an increase in the slopes of field EPSPs in hippocampal slices. We conclude that estrogen has opposing rapid actions on excitatory and inhibitory signaling, and that the net effect is an increase in the excitatory/inhibitory ratio.

Disclosures: J.C. Damborsky: None. **D.W. DuBois:** None. **A.S. Fincher:** None. **C. Roth:** None. **W.H. Griffith:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.16/F33

Topic: B.07. Synaptic Transmission

Support: KAKENHI 25293379

KAKENHI 25861764

Title: Reciprocal regulation of inhibitory synaptic transmission by nicotinic and muscarinic receptors in rat nucleus accumbens shell

Authors: K. EBIHARA[1,2], K. YAMAMOTO[1], K. UEDA[2], N. KOSHIKAWA[1], *M. KOBAYASHI[1];
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Abstract: Medium spiny neurons (MSNs) in the nucleus accumbens (NAc) are the principal neurons, whose neural activities are regulated by GABAergic inputs from MSN and fast-spiking interneurons (FSNs). Cholinergic interneurons play important roles in regulation of neural activities of MSNs; however, it is still unknown how acetylcholine modulates inhibitory synaptic transmission from MSNs/FSNs to MSNs. To clarify the cholinergic regulation of inhibitory synaptic transmission in the NAc comprehensively, it is necessary to discriminate the source of GABAergic inputs to the MSNs. In this study, we performed paired whole-cell patch-clamp recordings from MSNs and FSNs in rat NAc shell slice preparations and examined the nicotinic and muscarinic effects on unitary inhibitory postsynaptic currents (uIPSCs). Carbachol (1 μM) suppressed uIPSC amplitude by ~46% in MSN to MSN connections accompanying the increases in the paired-pulse ratio and failure rate, suggesting that acetylcholine reduces GABA release probability from synaptic terminals of MSNs. The carbachol-induced suppression of uIPSCs was antagonized by preapplication of atropine, and mimicked by pilocarpine (1 μM) but not by nicotine (1 μM). The analysis of coefficient of variation indicated that both carbachol and pilocarpine-induced uIPSC suppression were presumably mediated by presynaptic mechanisms. These results suggest that acetylcholine suppresses GABA release from MSNs via presynaptic muscarinic receptors. In contrast, FSN to MSN connections showed little effect of pilocarpine on uIPSC amplitude, whereas nicotine facilitated uIPSC amplitude by 30% with decreases in the failure rate and paired-pulse ratio suggesting that nicotine-induced uIPSC facilitation is mediated by presynaptic mechanisms. Miniature IPSC recordings suggest that muscarinic and nicotinic receptors reciprocally regulate the frequency of inhibitory inputs to the NAc MSNs. The present study suggests the contradictory role of presynaptic muscarinic and nicotinic receptors in modulating inhibitory synaptic transmission to MSNs in the NAc shell.

Disclosures: K. Ebihara: None. **M. Kobayashi:** None. **K. Yamamoto:** None. **N. Koshikawa:** None. **K. Ueda:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Support: NIH Grant DC005982

Stanford Graduate Fellowship

Lubert Stryer Stanford Interdisciplinary Graduate Fellowship

Title: GABAergic projection neurons gate odor-specific inputs to higher order neurons

Authors: *Y. LI[1,2], L. LIANG[3,5], C. POTTER[5,6,4], R. TSIEN[2,7], L. LUO[3,5];
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Abstract: An understanding of how brains perceive external stimuli requires functional analysis of the interplay between excitatory and inhibitory neurons along the sensory projection pathway. Using Drosophila olfactory system as a model system, we here characterize a unique group of inhibitory projection neurons (iPNs) which receive direct input from antennal lobe glomeruli and send output to the lateral horn, a higher brain center involved in regulation of innate behavior. Taking advantage of the newly developed Q-driver system and the genetically encoded calcium sensor GCaMP3, we performed two-photon live imaging of iPNs and their putative postsynaptic target vlpr neurons. We found food-related odorant responses were selectively suppressed by iPNs, whereas pheromone related signals were spared. Co-application of food and pheromone odorants did not elicit additional responses in pheromone channels different than pheromone alone, suggesting that the food-specific inhibition likely result from specific connectivity of iPNs, rather than a generalized inhibitory tone. Calcium responses of the presynaptic excitatory input from antennal lobe to lateral horn did not show any detectable suppression by iPNs, suggesting that the inhibition is likely postsynaptic. Our experiments reveal functional important GABAergic neurons that may provide specificity in inhibition to funnel specific olfactory information, such as food and pheromone signals, into distinct downstream circuits.

Disclosures: Y. Li: None. **L. Liang:** None. **R. Tsien:** None. **L. Luo:** None. **C. Potter:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.18/F35

Topic: B.07. Synaptic Transmission

Title: Imbalance of excitatory and inhibitory synaptic transmission lowers seizure threshold in RalBP1 hypomorphic mice

Authors: *S. H. YOON, Y.-S. BAE, K.-Y. PARK, M.-H. KIM;
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Abstract: Idiopathic epilepsy is believed to be caused by genetic defects. Mutations in genes leading to neuronal hyperexcitability or an imbalance between excitatory and inhibitory synaptic transmission seem to increase susceptibility to seizures by lowering the seizure threshold. However, the identity of these genes is unclear. In addition, the underlying mechanisms that link genetic background and altered seizure threshold are still poorly understood. In the present study, we investigated the role of RalBP1, a downstream effector of the small GTPases RalA and RalB, in seizure threshold. Reduced RalBP1 expression itself was not sufficient for seizure induction. However, the intraperitoneal injection of pentylenetetrazol (PTZ) induced more severe seizures in RalBP1 hypomorphic mice. Electrophysiological studies on hippocampal CA1 pyramidal neurons revealed decreased inhibitory synaptic transmission with normal excitatory synaptic transmission in RalBP1 hypomorphic mice. However, reduced RalBP1 function had no effect on neuronal excitability. The excitatory/inhibitory imbalance was associated with the loss of GABAergic interneurons in the CA1 subfield of the hippocampus. These results suggest that RalBP1 is essential for normal excitatory/inhibitory synaptic balance and seizure threshold.

Disclosures: S.H. Yoon: None. **Y. Bae:** None. **K. Park:** None. **M. Kim:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.19/F36

Topic: B.07. Synaptic Transmission

Support: NS022061

Title: Mechanisms of synaptic modulation by acetylcholine underlying acquisition and extinction of conditioned fear

Authors: *J. D. LEDERMAN, D. A. TALMAGE, L. W. ROLE;
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Abstract: Abnormal processing of sensory information underlies many cognitive disorders such attention deficit disorder, post-traumatic stress, and schizophrenia. The amygdala is especially important for integrating sensory information from many brain areas. In particular, afferents from cortical and thalamic sensory areas converge onto pyramidal neurons in the basolateral nucleus of the amygdala (BLA). In addition, the BLA is permeated by cholinergic fibers from the nucleus basalis of Meynert (NBM). These form few direct cholinergic synapses in the BLA and evidence suggests that acetylcholine primarily modulates other synapses in the BLA. Acetylcholine can dramatically alter glutamatergic transmission in the BLA and profoundly affects amygdala processing and behavior. This project asks how integration of convergent thalamic and cortical inputs to BLA is accomplished in the BLA and what role endogenous acetylcholine release plays in this integration.

To examine the behavioral role of endogenous ACh in the BLA, the nucleus basalis of Meynert is injected with an adeno-associated virus containing a double-inverted-floxed (DIO) oChIEF (an optimized version of channelrhodopsin 2), or a DIO-Halorhodopsin 3.0 construct in ChAT-Cre mice. The Cre/DIO system limits expression of the channel- or halo-rhodopsin to cholinergic cells while the afferents of these cells in BLA can be specifically stimulated or inhibited with high temporal precision by light of the appropriate wavelength. When these afferents are stimulated during training in a fear-conditioning paradigm, learned fear is enhanced and extinction of this fear is diminished. Conversely, inhibiting these afferents during training diminishes learned fear and enhances the extinction rate, supporting the role of ACh in memory acquisition and consolidation.

To understand the synaptic "rules" by which cholinergic modulation of glutamatergic synapses in the BLA occurs, patch-clamp electrophysiology is used in an acute slice preparation in conjunction with the optogenetic system described above. Stimulating electrodes are placed on the internal or external capsules to stimulate thalamic or cortical afferent fibers, respectively. Optogenetic stimulation of cholinergic afferents profoundly affects the magnitude and valence of plasticity at these glutamatergic synapses and suggests that endogenous ACh may differentially attune neurons to specific inputs. Moreover, ongoing experiments suggest that ACh may also influence the timing window in which these convergent inputs must coincide to induce plasticity.

Disclosures: J.D. Lederman: None. **L.W. Role:** None. **D.A. Talmage:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.20/F37

Topic: B.07. Synaptic Transmission

Support: NIH DA178188

Title: Presynaptic GABAb receptors silence excitatory synapses in neocortical networks

Authors: *J. URBAN CIECKO, E. FANSELOW, A. L. BARTH;
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Abstract: In superficial layers of the neocortex, about 10% of neocortical pyramidal neurons are connected to each other with strong and reliable synapses, displaying near-zero failure rates. However, this analysis has typically been carried out under high extracellular Ca levels and low network activity. In vivo, Ca levels are close to 1 mM and many cells, including inhibitory neurons, exhibit elevated spontaneous firing activity. Here we investigated the effect of physiological Ca levels and network activity on connection probability, strength, and synapse reliability between layer 2/3 pyramidal neurons. Under these conditions, connection probability is decreased by half, and failure rates are two-fold higher. Although higher failure rates can in part be attributed to lower Ca levels, we find that presynaptic GABAb receptors are tonically active during spontaneous network activity and that these receptors profoundly influence release probability. Pharmacological GABAb activation can effectively silence synapses, resulting in failure rates that approach 100%. Conversely, pharmacological GABAb blockade can suppress failures below baseline levels. These data suggest that neocortical networks may be dynamically rewired based upon presynaptic GABAb activation, and that this phenomenon may be state-dependent.

Disclosures: J. Urban Ciecko: None. **E. Fanselow:** None. **A.L. Barth:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Support: DA025676

DA026458

Title: Nicotine disinhibits pyramidal cells via activation of oriens/alveus interneurons in rat hippocampal CA1 region

Authors: *K. SUMIKAWA, S. NAKAUCHI;
Univ. California Irvine, Irvine, CA

Abstract: Previous results show that nicotine facilitates the induction of long-term potentiation in the hippocampal CA1 region, and suggest that this facilitation involves circuitry-dependent disinhibition of pyramidal cells. Here, we investigated disinhibition mechanisms by performing dual whole-cell recordings in hippocampal slices and identified two possible disinhibition pathways. Most of the oriens/alveus (O/A) interneurons, which have their axons originating from a proximal dendrite and exhibit a significantly larger fast afterhyperpolarization, showed tonic action potential discharges by bath application of nicotine. We first examined whether activation of these O/A neurons leads to inhibition of feedforward interneurons in the radiatum, which is a possible pathway for disinhibition of pyramidal cells. In some pair recordings, we found that current injection in O/A interneurons evoked inhibitory postsynaptic currents (IPSCs) in radiatum interneurons, indicating that they are functionally connected. In these pairs, bath application of nicotine in the presence of glutamate receptor antagonists caused depolarization and increased action potential firing in O/A interneurons, and also increased spontaneous IPSC frequency in radiatum interneurons. These findings provide a potential disinhibition pathway of CA1 pyramidal neurons by nicotine. We next examined whether activation of nicotine-responding O/A interneurons, which are connected to pyramidal cells, influences excitatory postsynaptic currents (EPSCs) evoked in pyramidal cells by Schaffer collateral stimulation. We found that the excitation of these O/A interneurons by current injection reduced the amplitude of EPSCs in connected pyramidal cells, but the amplitude of EPSCs increased to levels above the basal EPSC amplitudes when O/A interneuron stimulation was stopped. Although the underlying mechanism remains to be determined, the finding provides a novel disinhibition mechanism by nicotine. Thus, our results show that nicotine causes circuitry-dependent disinhibition of pyramidal cells via two different pathways, both of which require the activation of O/A interneurons.

Disclosures: K. Sumikawa: None. **S. Nakauchi:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.22/F39

Topic: B.07. Synaptic Transmission

Support: NRF of Korea 2012R1A1A4A01004566

Title: Dual mechanisms diminishing tonic $GABA_A$ inhibition of dentate gyrus granule cells in Noda epileptic rats

Authors: S. PANDIT[1], D. KIM[2], H. KIM[1], *J. PARK[1];
[1]Dept. of Physiol., [2]Dept. of Anat., Sch. of Medicine, Chungnam Natl. Univ., Daejeon, Korea, Republic of

Abstract: The Noda epileptic rat (NER), a Wistar colony mutant, spontaneously has tonic-clonic convulsions with paroxysmal discharges. In the present study, we measured phasic (I_{phasic}) and tonic γ-aminobutyric acid A ($GABA_A$) current (I_{tonic}) in NER hippocampal dentate gyrus granule cells (DGGCs) and compared the results with those of normal parent strain Wistar rats (WIS). I_{tonic}, revealed by a bicuculline-induced outward shift in holding current, was significantly smaller in NER than in WIS ($p < 0.01$). The frequency of inhibitory postsynaptic currents (IPSCs) was also significantly lower in NER than in WIS ($p < 0.05$), without significant differences in the IPSC amplitude or decay time between WIS and NER. I_{tonic} attenuation in NER was further confirmed in the presence of GABA transporter blockers, NO-711 and nipecotic acid, with no difference in neuronal GABA transporter expression between WIS and NER. I_{tonic} responses to extrasynaptic $GABA_A$ receptor agonists (THIP and DS-2) were significantly reduced in NER compared with WIS ($p < 0.05$). Allopregnanolone caused less I_{tonic} increase in NER than in WIS, while it prolonged the IPSC decay time to a similar rate in the two groups. Expression of the $GABA_A$ receptor δ subunit was decreased in the dentate gyrus of NER relative to that of WIS. Taken together, our results showed that a combination of attenuated presynaptic GABA release and extrasynaptic $GABA_A$ receptor expression reduced I_{tonic} amplitude and its sensitivity to neurosteroids, which likely diminishes the gating function of DGGCs and renders NER more susceptible to seizure propagation.

Disclosures: S. Pandit: None. **J. Park:** None. **D. Kim:** None. **H. Kim:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.23/F40

Topic: B.07. Synaptic Transmission

Title: Cholinergic modulation of intact CA3 circuits *In vivo*

Authors: *M. GRIGUOLI, S. ZUCCA, C. MULLE;
IINS, Interdisciplinary Inst. For Neurosci., Bordeaux Cedex, France

Abstract:
Hippocampus receives extensive cholinergic innervation from fibers mainly originating in the medial septal nucleus of the basal forebrain. Acetylcholine (ACh), released by cholinergic fibers,

targets nicotinic and muscarinic receptors distributed in all hippocampal regions including CA3 neurons, which are particularly involved in new memory encoding. The relevance of the hippocampus and the CA3 region for memory processes and the well known importance of the cholinergic system for cognitive functions make it necessary to better understand how the activity of CA3 circuits is controlled by ACh in the intact network of a mammalian brain. To address this question we used an optogenetic approach, to selectively control the activity of cholinergic neurons, combined with *in vivo* recordings from CA3 pyramidal neurons. Double-floxed inverted open reading frame (DIO) viral vectors were used to target the expression of ChR2-EYFP to the cholinergic neurons of the *medial septum* in ChAT-Cre mice. To test the functionality of ChR2 we first performed whole cell recordings from EYFP positive cholinergic cells in acute brain slices. Brief pulses of blue light (0.5-1 ms; 470 nm) reliably induced action potentials at different frequencies of stimulation, indicating that this population of cholinergic neurons can be optically controlled. To study how the activation of the cholinergic neurons modulate CA3 network activity in the anesthetized mouse, an optical fiber was acutely implanted in the *medial septum* to deliver optical stimulation (1-5 ms, 470nm). We then recorded multi unit activity and oscillatory network activity (theta and gamma bands) in basal conditions and after light-mediated cholinergic stimulation. Activation of the cholinergic system increased the firing frequency of CA3 neurons and was associated with a consistent increase in the power of theta oscillations. Further studies at the single cell level will allow us to better characterize the effect of cholinergic activation on intrinsic membrane properties and synaptic inputs. This study will gain new insights on the functional role of ACh in modulating CA3 circuits involved in the rapid encoding of new information.

Disclosures: M. Griguoli: None. **S. Zucca:** None. **C. Mulle:** None.

Poster

230. **Synaptic Transmission: Modulation I**

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National Science Council (NSC101-2321-B-010-024)

National Science Council (NSC100-2320-B-010-014-MY3)

Title: Wiring principles in lateral subdivision of central amygdala

Authors: *W. S. HO, C.-C. LIEN;
Inst. of Neurosci., Natl. Yung-Ming Univ., Taipei, Taiwan

Abstract: Abstract
The amygdala is a key brain region for fear acquisition and expression. The lateral subdivision of the central amygdala (CeL), which comprises over 90% of GABAergic neurons, provides tonic inhibition onto the final output of the amygdala - the medial subdivision of the central amygdala (CeM) and thus controls fear expression. However, the CeL contains multiple neuronal subtypes and the functional connections between CeL neurons remain unclear. To answer this question, we performed whole-cell patch-clamp recordings from CeL neurons and found at least three different types of CeL neurons on the basis of their electrophysiology properties: late spiking (LS), regular spiking (RS) and low-threshold bursting (LTB) neurons. Among them, 90% of neurons were LS and RS neurons. Simultaneous pair- or triple-recordings from CeL neurons showed that temporal dynamics of GABA transmission within the CeL was target cell-specific. Specifically, synaptic transmission at LS-to-RS or RS-to-LS neuron output synapses exhibited activity-dependent depression, whereas the output synapses of LS-to-LS or RS-to-RS neurons were relatively insensitive to presynaptic activity or exhibited use-dependent facilitation. In addition to mutual inhibition, we also found that both LS and RS neurons exhibited autapses, which sent their axons back to modulate their own activity. Our preliminary results suggest that the diversity of GABAergic neurons and synapses may enable combinatorial inhibitory effects in the CeL and thus modulate fear expression.

Disclosures: W.S. Ho: None. **C. Lien:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.25/F42

Topic: B.07. Synaptic Transmission

Title: GABAergic dysfunction in anterior cingulate cortex of heterozygous BRD1 knockout mice

Authors: *I. V. VARDYA[1], J. H. CHRISTENSEN[1,2], P. QVIST[1,2], M. NYEGAARD[1,2], O. MORS[2,3], K. JENSEN[1], A. D. BØRGLUM[1,2,3];
[1]Dept. of Biomedicine, Aarhus Univ., Aarhus, Denmark; [2]The Lundbeck Fndn. Initiative for Integrative Psychiatric Res., iPSYCH, Denmark; [3]Ctr. for Psychiatric Research, Aarhus Univ. Hosp., Risskov, Denmark

Abstract: Anterior cingulate cortex (ACC) is a brain region that is critical for higher cognitive functions as attention, control of behavior and remote memory expression. Synchronization of pyramidal neurons of ACC is essential for the performance of neuronal circuits and dependent on activity of local GABAergic interneurons (Goldman-Rakic 1995). Alterations in GABA-mediated signaling in ACC were shown to be implicated in pathology of psychiatric disorders. Here, we investigated properties of GABA-mediated synaptic transmission in ACC in a new genetically modified mouse model of schizophrenia and bipolar disorder – heterozygous BRD1(R) knockout mice. The *BRD1 gene*, encoding a regulator of transcription - the bromodomain-containing protein1 (BRD1), was proposed to be susceptibility gene for schizophrenia and bipolar disorder (Severinsen et al. 2006; Nyegaard et al. 2010). Experiments were performed on adult male mice, BRD1(R) and BRD1(WT) littermates in the age range from 7 to 8 weeks. In acute coronal brain slices, whole-cell patch-clamp recordings from pyramidal neurons layers 2/3 of ACC were done. Pyramidal neurons were voltage-clamped at V_{hold} of -70mV and $GABA_A$ mediated inhibitory postsynaptic currents (IPSCs) were recorded in the presence of kynurenic acid, bloker of ionotropic glutamate receptors. We found 40% deficit in activity dependent GABAergic neurotransmission in ACC of BRD1(R) mice. Frequency of sIPSCs was 17.2 ± 1.6 Hz (n = 24) in BRD1(WT) and 10.1 ± 1.2 Hz (n = 21, $P < 0.01$) in BRD1(R) group. We repeated the experiments in the presence of the Na^+ channel blocker tetrodotoxin (1 μM) to isolate miniature IPSCs (mIPSCs). Frequency of mIPSCs was 8.2 ± 0.9 Hz (n = 8) in WT and 6.0 ± 0.7 Hz (n = 12) in R mice. There was no difference in mIPSCs mean amplitude, rise time or decay time between BRD1(WT) (39.9 ± 1.9 pA; 295.2 ± 15.0 μs; 4.1 ± 0.2 ms; n=8) and BRD1(R) mice (36.7 ± 2.0 pA; 298.7 ± 12.2 μs; 4.4 ± 0.2 ms; n = 12), indicating similar density and composition of synaptic $GABA_A$ receptors of pyramidal neurons of ACC. Interesting, a similar decrease in GABAergic inhibition was found in pyramidal neurons of somatosensory cortex, where significant reduction in GABA-mediated neurotransmission (from 12.5 ± 1.6 Hz (n = 11) in BRD1(WT) to 7.5 ± 0.9 Hz (n = 17 $P < 0.05$) in BRD1(R)) and in release probability of perisomatic GABAergic synapses was found. The data propose similar origin of GABAergic dysfunction throughout the cortical networks in the BRD1(R) mice. Deficit in GABA-mediated synaptic transmission in ACC of BRD1(R) mice may lead to altered synchronization of the cortical network and be involved in mechanisms, underlying behavior and cognitive alterations found in this model.

Disclosures: I.V. Vardya: None. **J.H. Christensen:** None. **P. Qvist:** None. **M. Nyegaard:** None. **O. Mors:** None. **K. Jensen:** None. **A.D. Børglum:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.26/F43

Topic: B.07. Synaptic Transmission

Support: National Science Foundation CAREEER Award 0952686

NIH Grant T32GM007839-33

Title: Acetycholine reorganizes functional neocortical circuitry to favor feedforward thalamocortical information flow into superficial lamina

Authors: *M. J. RUNFELDT[1], J. N. MACLEAN[1,2];
[1]computational neuroscience, [2]Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Acetylcholine (ACh) has hetereogeneous effects on individual cortical neurons, yet has consistently been shown to increase intertrial reliability, enhance stimulus discriminability, and reduce pairwise activity correlations. These changes have been suggested to underlie to the attentional improvement of stimulus discriminability in awake behaving animals. However, it remains unclear how heterogeneous single cell changes driven by ACh receptor activation produce these changes in the population statistics. We address this question directly at the microcircuit level by sampling 600 +/- 165 neurons in a 1.1 mm diameter field of view using high speed multiphoton calcium imaging (15 +/-5 Hz sampling) in combination with patch clamp physiology.
We found that ACh (50μM) modifies spontaneous and thalamically-evoked activity differently. ACh causes a reduction in both the reliability and the numbers of recruited neurons in activity that spontaneously emerges intracortically. In contrast, more neurons are active in superficial lamina and demonstrate enhanced reliability when activity is thalamically evoked in the presence of ACh. We find that the majority of neuronal pairs, which have modest activity correlations and low joint probability of firing, are further reduced in the presence of ACh. In contrast, strong correlational structures, such as those activated by thalamic input, are strengthened. We also find that the neuronal pairs most correlated with ACh are further apart in temporal recruitment and geometric space. This places network dynamics within a regime that permits increased reliability of transmission of afferent input throughout local neocortical circuitry.

Disclosures: M.J. Runfeldt: None. **J.N. MacLean:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.27/F44

Topic: B.07. Synaptic Transmission

Support: JST,CREST

Comprehensive Brain Science Network (CBSN)

Title: Distribution of GAD65 and GAD67 immunoreactive somata in the mouse cortex: Classification using molecular markers

Authors: *H. MIWA[1,2], M. WATANABE[3], Y. YANAGAWA[1];
[1]Genet. & Behavioral Neurosci., Gunma Univ., Maebashi, Gunma, Japan; [2]JST, CREST, Tokyo, Japan; [3]Hokkaido Univ. Sch. of Med., Sapporo, Japan

Abstract: Inhibitory neurons play important roles in the regulation and stabilization for neural network activities and are essential for brain functions, including cognition, perception, movement, and emotion. γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. GABA is synthesized from glutamate by two glutamate decarboxylase (GAD) isoforms, GAD65 and GAD67. The two GAD isoforms in somata are known to be heterogeneous among different subpopulations of GABAergic neurons. However, which subpopulation of GABAergic neurons exhibits somata labeling for the GADs remains unknown. Here, we examined the immunoreactivities against two GAD isoforms, GAD65 and GAD67, in somata of molecular markers [parvalbumin (PV), somatostatin (SST), vasoactive intestinal peptide (VIP), and reelin]-positve GABAergic neurons in the mouse cortex. The somata of GABAergic neurons containing PV and reelin showed more intense immunoreactivities against GAD67 than SST- and VIP-containing GABAergic neurons. These results indicate that different subpopulations of GABAergic neurons exhibit distinct expression levels of two GADs. In addition, they suggest that altered expression levels of GAD67 or GAD65, as reported in several psychiatric diseases including schizophrenia, would have GABAergic neuron subpopulation-specific consequences on the regulation of GABAergic neurotransmission.

Disclosures: H. Miwa: None. **Y. Yanagawa:** None. **M. Watanabe:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.28/F45

Topic: B.07. Synaptic Transmission

Support: NSERC

FRQS

Title: Carbachol induces a relative facilitation of intracellular EPSPs during short trains of theta- and gamma-frequency stimulation of the entorhinal cortex

Authors: *D. W. SPARKS, C. A. CHAPMAN;
Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: The superficial layers of the entorhinal cortex provide the hippocampus with much of its cortical sensory input, and the parasubiculum sends its single major output projection to layer II of the entorhinal cortex. The parasubiculum is therefore in a unique position to contribute to the modulation of entorhinal inputs to the hippocampal formation. Both the parasubiculum and entorhinal cortex receive cholinergic inputs that contribute to theta- and gamma-frequency EEG activities that are expressed as animals explore their environment. We previously found, using in vitro field potential recordings, that application of the cholinergic agonist carbachol, although suppressing the overall amplitude of evoked responses in parasubicular inputs to layer II of the medial entorhinal cortex, results in a relative facilitation of responses evoked during short 5-pulse trains of stimulation delivered at theta (10Hz) and gamma (33Hz) frequencies. This facilitation effect was dependent on M1 muscarinic receptors, was partially blocked by the NMDA receptor antagonist APV, and was completely blocked by ZD7288, which blocks the hyperpolarization-activated current Ih. The current study investigated the relative facilitation effect induced by carbachol in intracellularly recorded EPSPs during theta- and gamma-frequency stimulation at resting membrane potential and at hyperpolarized potentials. Whole-cell patch clamp recordings were obtained from layer II neurons of the medial entorhinal cortex in acute brain slices, and responses were evoked by stimulation of the parasubiculum using 5-pulse trains of stimulation at 10 or 33Hz. Hyperpolarization was associated with a large increase in EPSP amplitude despite no substantial change in input resistance, suggesting an effect of increased driving force on the EPSP. Similar to effects observed previously, intracellular responses during trains were reduced in normal ACSF (with a tendency for greater decline at hyperpolarized potentials), and carbachol led to a relative facilitation of the amplitude of later pulses in the trains for both theta- and gamma-frequency stimulation. Larger and more variable facilitation effects were observed at rest versus at hyperpolarized potentials. These results confirm that, even though carbachol suppresses the amplitude of single EPSPs in the parasubiculo-entorhinal pathway, carbachol also induces a relative facilitation of intracellular EPSPs evoked during short 5-pulse theta- and gamma-frequency trains. This novel finding suggests that repetitive synaptic inputs at these frequencies may be relatively well maintained as animals move about and explore their environment.

Disclosures: D.W. Sparks: None. **C.A. Chapman:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.29/F46

Topic: B.07. Synaptic Transmission

Support: R01 MH061059

Title: Regulation of excitatory synaptic transmission at the hippocampal slices by synapsin II and Rab3a

Authors: *K. J. QUINONES, P. FELICIANO, M. BYKHOVSKAIA;
NEUROSCIENCE, Univ. Central Del Caribe, BAYAMON, Puerto Rico

Abstract: Synapsins are a family of synaptic vesicle phosphoproteins involved in the regulation of synaptic transmission. In humans, mutation on synapsin genes has been associated to the pathogenesis of epilepsy, while the deletion of synapsin genes has been implicated to induce epileptic seizures in mouse. The deletion of Rab3A, a synapsin binding partner involved in the vesicle mobilization, rescues the seizure activity observed at synapsin II deleted mice (SynII(-)). To understand how the interaction between these two proteins regulates synaptic activity, we investigated excitatory synaptic transmission at Syn II(-), Rab3a(-), and Syn II(-)/Rab3a(-) double knockout (DKO) mice. We have employed whole cell recordings from CA1 pyramidal neurons at hippocampal brain slices, while stimulating Schaffer collaterals at different current intensities. We detected a significantly increased synaptic activity at SynII(-) slices, and a significantly decreased synaptic activity at Rab3a(-) slices, while the activity at SynII(-)/Rab3A(-) DKO slices was not different from wild type (WT). Since reduced synaptic transmission is usually associated with an increase in pair-pulse facilitation (PPF), we investigated PPF in all the strains. The PPF at Rab3a(-) synapses was significantly increased, in agreement with the reduced synaptic activity. This result suggests that basal glutamatergic transmission at Rab3a(-) synapses may be diminished.

Disclosures: K.J. Quinones: None. **P. Feliciano:** None. **M. Bykhovskaia:** None.

Poster

230. **Synaptic Transmission: Modulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 230.30/G1

Topic: B.07. Synaptic Transmission

Support: DFG Hu797/7-1

Title: Lethal phenotype of conditional VIAAT-knock-out in glycinergic neurons

Authors: J. RAHMAN[1], C. SCHNELL[1], *V. EULENBURG[2], S. M. WOJCIK[3], S. HÜLSMANN[1];
[1]Inst. for Neurophysiol. and cellular Biophysics, Goettingen, Germany; [2]Univ. of Erlangen, Inst. for Biochem. and Mol. Med., Erlangen, Germany; [3]Max-Planck-Institute of Exptl. Med., Göttingen, Germany

Abstract: The vesicular inhibitory amino acid transporter (VIAAT) is required for synaptic vesicle filling with GABA and/or glycine. To test the effect of a VIAAT knockout in glycinergic neurons we crossed floxed VIAAT mice to a BAC-transgenic GlyT2-Cre mouse line (Tg(Slc6a5-icre)121Veul). Knockout embryos (E18.5) delivered by caesarian section had a severe phenotype, with omphalocele, cleft palate, and respiratory failure. Electrophysiological recordings from hypoglossal neurons revealed the absence of spontaneous inhibitory postsynaptic currents (IPSCs), while evoked GABAergic inhibitory post-synaptic currents (eIPSCs) were unchanged and evoked glycinergic eIPSCs were decreased. The resting membrane potential of knockout neurons was slightly depolarized as compared to neurons from control littermates. Furthermore, immunohistochemical VIAAT-staining showed strongly reduced expression in the brainstems of knockout embryos. These results demonstrate that deletion of VIAAT in GlyT2-Cre-expressing neurons also strongly effects GABAergic transmission and suggest a large overlap of these two inhibitory neuron populations during embryonic development.

Disclosures: J. Rahman: None. **C. Schnell:** None. **V. Eulenburg:** None. **S.M. Wojcik:** None. **S. Hülsmann:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.01/G2

Topic: B.07. Synaptic Transmission

Support: PICT-2008-2019

Title: Lactic acid modulates excitatory synaptic transmission at the calyx of Held

Authors: *O. D. UCHITEL[1], C. GONZALEZ INCHAUSPE[2], M. N. DI GUILMI[2];
[1]FBMC, IFIBYNE UBA CONICET, Buenos Aires 1428, Argentina; [2]IFIBYNE-CONICET, FCEyN, Univ. of Buenos Aires, Buenos Aires, Argentina

Abstract: Lactic acid is produced by anaerobic metabolism during physiological and pathological conditions in different sites: from muscle to brain. As a consequence, the pH in-vivo can fall as low as 6.5. The mechanism of action is still unclear but it was proposed an indirect role that would increase the activity of an acid-sensing ion channels (ASICs) and a direct role as a chelator of extracellular divalent ions (Immke and McCleskey, 2001). In this work we studied the effect of lactic acid on synaptic transmission using the calyx of Held-MNTB giant excitatory glutamatergic synapse in the mammalian central nervous system. Lactic acid (15 mM) enhanced ASIC currents evoked in postsynaptic MNTB neuron by a rapid drop in extracellular pH (reversible after washout). In addition, lactic acid decreased EPSC amplitudes as well the depression rate during high frequency trains at 100 and 300 Hz. The addition of extra calcium and magnesium to aCSF, reaching values of 2.35 Ca^{2+}/1.12 Mg^{+} (Immke and McCleskey, 2001), partially restored the original short term depression feature. These data suggest a direct role of lactic acid on synaptic transmission in addition to its role as a chelator of divalent ions and as modulator of postsynaptic ASIC channels.

Disclosures: O.D. Uchitel: None. **C. Gonzalez Inchauspe:** None. **M.N. Di Guilmi:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

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Program#/Poster#: 231.02/G3

Topic: B.07. Synaptic Transmission

Support: NIH NIAID IAA #AOD12058-0001-0000

DTRA-JSTO Grant CBM.THRTOX.01.10.RC.021

Title: Network reponses to botulinum neurotoxin in synaptically coupled central nervous system neurons

Authors: *P. H. BESKE, K. S. HUBBARD, I. M. GUT, M. E. LYMAN, P. M. MCNUTT;
US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: Peripheral administration of *Clostridium botulinum* neurotoxins (BoNTs) is indicated for an increasing number of cosmetic and therapeutic applications. While much research effort has focused on BoNT intoxication of the neuromuscular junction, relatively little is known about the effect of BoNT on central synapses. In light of recent proposals suggesting therapeutic value for the central injection of BoNT, a functional determination of the consequences of intoxicating a networked population of central neurons remains a critical need. Therefore, to characterize the effect of BoNT intoxication on central synapses, we performed a multi-technique approach utilizing networked populations of murine embryonic stem cell-derived glutamatergic (90%) and GABAergic (10%) neurons (ESNs) as a central nervous system model. Whole-cell patch clamp electrophysiology revealed that ESNs develop mature electrical responses and form a complex synaptically coupled network after 18 days *in vitro* (DIV). Treatment of DIV 21+ ESNs with 2 pM of BoNT serotype A (BoNT/A) resulted in decreased synaptic signaling within 3 h, as determined by significant reductions in spontaneous action potentials (APs), excitatory post-synaptic potentials (EPSPs), and miniature excitatory post-synaptic currents (mEPSCs). ESNs were found to be highly sensitive to BoNT/A, with significant inhibition of APs, EPSPs, and mEPSCs at 24 h with doses as low as 0.1 mouse lethal units (0.067 pM). Given a calculated EC50 at 24 h of 0.81 pM using immunoblot detected cleavage of SNAP-25, measured inhibition of synaptic activity may provide a more sensitive metric of BoNT intoxication. Interestingly, early evidence suggests that treatment of ESNs with very low doses of BoNT/A perturbs the excitatory/inhibitory balance and elicits an epileptiform network response. Collectively, these studies functionally elucidate central network consequences of BoNT intoxication in a highly sensitive *in vitro* model system. This approach suggests that electrophysiological characterization of trans-synaptic activity may comprise a novel, rapid screen for the presence of functional toxin in forensic, pharmaceutical, environmental and/or food samples, as well as provide an *in vitro* model for central network research involving BoNT-induced synapse silencing. *Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.*

Disclosures: P.H. Beske: None. **K.S. Hubbard:** None. **I.M. Gut:** None. **M.E. Lyman:** None. **P.M. McNutt:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.03/G4

Topic: B.07. Synaptic Transmission

Support: USPHS Grant MH083729

Title: Modulation of glutamate and dopamine levels in the prefrontal cortex by intra-cerebellar kynurenic acid infusion in the rat

Authors: *H.-Q. WU, R. SCHWARCZ;
Maryland Psych Res. Ctr, Univ. Maryland Sch. Med., Baltimore, MD

Abstract: Kynurenic acid (KYNA) is an astrocyte-derived, neuromodulatory tryptophan metabolite, which can act as an antagonist of both α7 nicotinic acetylcholine and NMDA receptors. KYNA levels are increased in several brain regions of individuals with schizophrenia (SZ) and may play a role in the cognitive impairments seen in the disease. A dysfunctional, cross-hemispheric connectivity between the cerebellum and the medial prefrontal cortex (mPFC) has been repeatedly proposed to be involved in the cognitive and affective deficits seen in SZ and other psychiatric diseases (Biol. Psychiatry, 64:81, 2008; Synapse, 65:1204, 2011). The present study was designed to examine the effects of cerebellar KYNA fluctuations both locally (i.e. within the cerebellum) and in the distant mPFC. To this end, extracellular levels of KYNA and glutamate in the cerebellum, and dopamine and glutamate in the contralateral mPFC, were determined in microdialysate samples obtained in freely moving rats. Locally, application of KYNA's bioprecursor kynurenine (0.5-10 µM) into the Purkinje cell layer by reverse dialysis produced the expected concentration-dependent increase in KYNA levels, and a corresponding reduction in glutamate, measured in the same microdialysate fraction. In contrast, intra-cerebellar infusion of (*S*)-4-(ethylsulfonyl)benzoylalanine (*S*-ESBA; 0.5-5 mM), a specific inhibitor of KYNA biosynthesis (ChemMedChem 1:528, 2006), induced a concentration-dependent reduction in KYNA and an increase in glutamate in same dialysate sample. Experimental manipulations of KYNA in the cerebellum resulted in qualitatively different consequences in the mPFC. Thus, an intra-cerebellar infusion of KYNA (10 µM, 10 µl for 2 hrs) induced significant peak increases in the extracellular levels of both dopamine (+50%) and glutamate (+75%) in the mPFC. This effect was duplicated by an intra-cerebellar infusion of kynurenine (5 µM, 10 µl for 2 hrs). In contrast, an intra-cerebellar infusion of *S*-ESBA (5 mM, 10 µl for 2 hrs) caused a decrease in extracellular dopamine (-20%) and glutamate (-30%) levels in the contralateral mPFC. Notably, an intra-cerebellar infusion of the specific glycine$_B$/NMDA receptor antagonist 7-Cl-kynurenic acid (10 µM, 10 µl for 2 hrs) affected neither extracellular dopamine nor glutamate levels in the mPFC, indicating that cerebellar NMDA receptors are not involved in the distant effects of KYNA described here. Current studies in the laboratory interrogate the characteristics of the polysynaptic connections that link fluctuations in cerebellar KYNA with prefrontally mediated behaviors, and the role of these phenomena in pathological conditions.

Disclosures: H. Wu: None. **R. Schwarcz:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

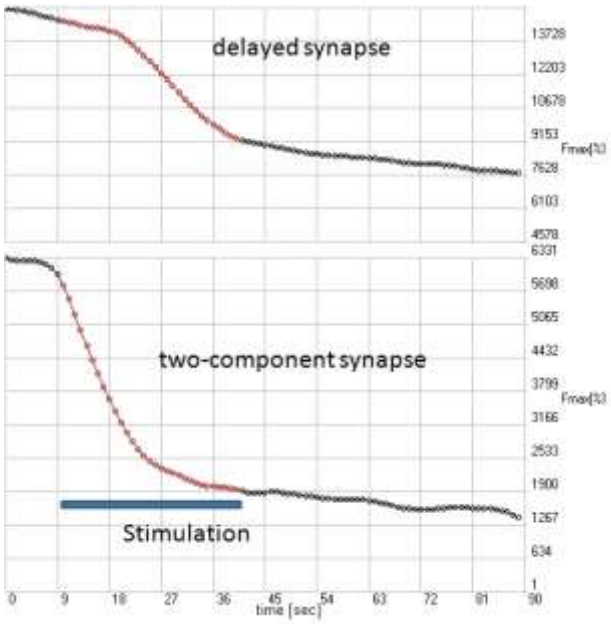
Program#/Poster#: 231.04/G5

Topic: B.07. Synaptic Transmission

Title: Fluoxetine alters the mode of exocytosis in kinetically distinguished synaptic subtypes

Authors: *A. W. HENKEL[1], O. WELZEL[2];
[1]Physiol., Kuwait Univ., Safat, Kuwait; [2]Dept. of Psychiatry and Psychotherapy, Univ. of Erlangen-Nuremberg, Erlangen, Germany

Abstract: Neural transmission requires quantitative modulation of transmitter release and temporal coordination of information flow. Here we describe that the onset of synaptic exocytosis, monitored with FM1-43, can be significantly delayed. Hippocampal neurons from newborn rats were cultured in vitro until they obtained functionally mature synapses. Synaptic vesicles were fluorescently labeled, using 600 pulses at 30 Hz to label the whole recycling pool. After thorough wash-out, boutons were destained in two consecutive stimulation cycles, where the first served as internal control. Neurons were either treated with 1 µM fluoxtine or control solution during the second cycle and kinetic parameters from both cycles were compared. Mean exocytosis of FM followed roughly a single exponential decay curve during the stimulation period. Detailed analysis, however, revealed that the general synaptic population was composed of at least 4 kinetically distinguished exocytosis subtypes 1. single-exponential type, 2. delayed type, 3. two-component type and linear type. FM-exocytosis kinetics were significantly altered by application of fluoxetine during the second stimulation cycle, which decreased the number of delayed synapses by more than 50 % and increased the speed of synaptic exocytosis significantly by rising the percentage of fast two-component synapses. Synapses in control experiments showed the opposite effects when they were stimulated in a second stimulation cycle. Exocytosis in synapses, expressing synaptopHlourin as a reporter flurophore, was never delayed after start of stimulation. Considering earlier findings on synaptic release of FM1-43, our results suggest that the mode of exocytosis is switched between full vesicle fusion and “kiss-and-run” within the same synapse. The delay of FM release is due to “kiss-and-run” type of exocytosis, because the dye cannot leave a vesicle through a narrow fusion pore. Both, H+ ions and neurotransmitter molecules, however, can leave the vesicle lumen through a pore instantly, and therefore allow synaptopHlourin to monitor exocytosis.



Disclosures: A.W. Henkel: None. **O. Welzel:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.05/G6

Topic: B.07. Synaptic Transmission

Support: NIGMS 1P20GM103653 - 01A1

Title: Role of a D2-like auto-receptor in modulating dopamine release in C.elegans and the potential co-transmission of dopamine with glutamate

Authors: *H. S. DHILLON[1], R. FORMISANO[1], M. MERSHA[2], R. KING[2], P. HAN[2], J. SINGH[2];
[1]Biol. Sci., [2]Delaware State Univ., DOVER, DE

Abstract: Dopamine is known to influence a wide range of neural processes including motor control, learning, memory and seeking behavior. Several neurological disorders are caused by dopamine imbalances, either due to low dopaminergic transmission (PD or attention deficit-hyperactive disorder, ADHD) or high dopamine levels (schizophrenia). At the cellular level there is degeneration of dopaminergic neurons in the ventral tegmental area of the midbrain of Parkinson's disease patients that leads to decreased levels of the monoamine neurotransmitter

dopamine that may involve D-2 like auto-receptors. We are using the *Caenorhabditis elegans* model to understand the auto-receptor functional component of the DOP-2 receptor in modulating dopamine levels in the synaptic cleft. In *C. elegans*, the D2-like dopamine receptor DOP-2 is expressed in all eight of its dopaminergic neurons and has been proposed to act as an auto-receptor that modulates dopamine release during the process of associative learning in the nematode. Four dopamine receptors have been identified in the *C. elegans* genome: DOP-1, DOP-2, DOP-3, and DOP-4. Their gene products are representative of two classes of dopamine receptors found in mammals: the D1-like and the D2-like receptors. Binding of dopamine to its G-protein coupled seven-transmembrane dopamine receptors can trigger antagonistic signal transduction cascade. Our interest is to understand the auto-receptor functional components of DOP-2 in regulating dopamine release. Recent work in our lab has shown that DOP-2 physically interacts with GPA-14, an inhibitory G-alpha subunit, and that both *dop-2* and *gpa-14* deletion mutants habituate at a significantly faster rate as compared to wild-type worms. Moreover, *gpa-14* deletion mutants have also shown associative learning deficits similar to those reported for *dop-2* in previous studies. In order to characterize the downstream molecular components of the DOP-2 auto-receptor function we are characterizing other mutants in TRP-N gene. In addition, we have quantified differential levels of alternatively spliced forms of the *dop-2* transcript in order to dissect function(s) of individual splice variants. Considering that the vulnerability of dopaminergic neurons in midbrain of PD patients is a likely function of excitotoxic death due to failure to regulate glutamate levels, we are also examining potential co-transmission of dopamine with glutamate and if D2 auto-receptors play a role in modulating co-release of the two neurotransmitters.

Disclosures: H.S. Dhillon: None. **R. Formisano:** None. **M. Mersha:** None. **R. King:** None. **P. Han:** None. **J. Singh:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.06/G7

Topic: B.07. Synaptic Transmission

Support: NIH Grant R00NS051401

Title: Effects of action potential waveforms and the depolarizing afterpotential on calcium channel activity in the calyx of Held

Authors: *K. G. PARADISO, S. CLARKE, A. KISNER, B. CROWELL;
Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ

Abstract: At the calyx of Held nerve terminal, action potential waveforms can be used to activate calcium channels and trigger neurotransmitter release. Blockers for voltage-gated sodium and potassium channels are used to isolate calcium channel activity, and low resistance pipettes placed near the center of the calyx are used to optimize the ability to rapidly voltage-clamp the calyx. Using paired recordings, studies by others have clearly demonstrated that small changes in the kinetics or amplitude of the action potential can have large effects on the amount of neurotransmitter release. Following each action potential in the calyx, there is a depolarizing afterpotential (DAP) that affects the membrane potential reached by the repolarizing phase of the action potential. However, little is known about the effects of the DAP on calcium channel response. In order to test this, we have used a series of different action potential waveforms with and without this depolarizing afterpotential to activate calcium channels in the presynaptic terminal. At this time, the presence or absence of the DAP has not indicated any appreciable difference in the amount of calcium channel activity the action waveforms generate. However, since the presynaptic terminal is sensitive to very small changes in calcium, we are now investigating effects on exocytosis by using paired recordings or capacitance measurements to determine if the depolarizing afterpotential alters neurotransmitter release. In addition, to further determine how the shape of the action potential affects the calcium channel response, we have tested a series of different action potential waveforms that have the same total area, but vary in their rise and decay time. We started with a symmetrical waveform that has a rise time and decay time of 0.5 msec giving a total duration of 1 msec. Our results for a symmetrical waveform indicate that activation of calcium channels appears to begin during the peak of the action potential, continues during the repolarization phase and is largely complete by the end of the action potential. This finding agrees with similar experiments done by other labs. Of the waveforms we have tested that have identical areas, it is evident that a square waveform, although physiologically impossible, is the most efficient at activating calcium channels. Still, due to the short duration, even a square waveform will only activate ~50% of the peak calcium channel response. Finally, we are also investigating the extent to which various action potential waveforms affect the onset and recovery of calcium channel inactivation.

Disclosures: K.G. Paradiso: None. **S. Clarke:** None. **A. Kisner:** None. **B. Crowell:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.07/G8

Topic: B.07. Synaptic Transmission

Support: American Heart Association Grant 11GRNT7890031

NIH Grant R01 NS052446

Title: Potentiation of adrenal catecholamine secretion by prostaglandin E_2: A novel role for "inhibitory" G proteins?

Authors: *R. L. BRINDLEY[1], M. L. JEWELL[2], K. P. M. CURRIE[3];
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Abstract: Adrenal chromaffin cells are an important neuroendocrine component of the sympathetic nervous system that release catecholamines, neuropeptides, and other hormones to help maintain homeostatic functions and appropriate responses to acute stress. G protein coupled receptors (GPCRs) integrate a variety of autocrine / paracrine signals to precisely control catecholamine exocytosis. Systemic immune challenge or inflammatory cytokines are thought to boost local production of prostaglandin E_2 (PGE_2) in the adrenal gland where mRNA for all four EP receptors (EP1 - EP4) is expressed. Previously we showed that PGE_2 inhibits Ca_v2 voltage-gated calcium channel currents (I_{Ca}) and exocytosis (changes in membrane capacitance) evoked by brief depolarizing stimuli in mouse chromaffin cells (*Mol. Pharm. 2011, 79: 987-996*). This pathway is mediated by G protein βγ subunits (Gβγ) liberated from $G_{i/o}$-type G proteins downstream of EP3 receptor activation by PGE_2. Here we show that during sustained stimulation to mimic acute stress, this canonical inhibition transitions to a novel potentiation of evoked catecholamine secretion. Catecholamine secretion was evoked by two rounds of stimulation with 30 mM KCl and detected using carbon fiber amperometry. In chromaffin cells isolated from wild-type mice, PGE_2 produced a robust potentiation of secretion evoked by KCl that was primarily due to a significant increase in the number of vesicular fusion events (amperometric spikes). The potentiation was abolished in cells treated with pertussis toxin indicating involvement of $G_{i/o}$-type G proteins. Further, two distinct antagonists of Gβγ signaling (gallein or anti-Gβγ phosducin-like C terminus peptide) blocked the PGE_2 mediated potentiation of catecholamine secretion. The potentiation was also prevented by a selective antagonist of the EP3 receptor (DG-041), and not seen in cells isolated from EP3 knockout mice. Thus, during brief stimuli, EP3 receptors suppress secretion through inhibition of I_{Ca}, but during sustained stimuli EP3 receptors potentiate evoked catecholamine secretion through a distinct pathway which also involves Gβγ subunits liberated from $G_{i/o}$-type G proteins. Taken together, our data reveal a rich, context-dependent modulation of catecholamine secretion by the inflammatory mediator PGE_2, and identify a novel signaling pathway through which "inhibitory" G-proteins can potentiate neuroendocrine hormone secretion.

Disclosures: R.L. Brindley: None. **M.L. Jewell:** None. **K.P.M. Currie:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.08/G9

Topic: B.07. Synaptic Transmission

Support: NSYSUKMU 2013-P005

Title: Mechanism of H_2O_2-induced facilitation on synaptic activity at developing neuromuscular synapse

Authors: *J.-C. LIOU;
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Abstract: Although hydrogen peroxide (H2O2), a membrane-permeable reactive oxygen species, is better known for its cytotoxic effect, it has recently been suggested that it is released during neuromuscular activity and plays a negative feedback modulator on synaptic transmission. Here we test the role of H2O2 on synaptic transmission in developing Xenopus neuromuscular synapse by using whole-cell patch clamp recording. Bath application of H2O2 dose-dependently increases the spontaneous ACh secretion. Treatment the culture with either catalase or N-acetylcysteine has no significant on basal spontaneous synaptic neurotransmitter release, indicating H2O2 is not a retrograde factor, which might drive the machinery of neurotransmitter release. We next test the origin of H2O2-induced synaptic facilitation. The H2O2-induced synaptic facilitating effect was hampered while catalase was loading into myocyte through recording pipette, suggesting postsynaptic myocyte play crucial role in H2O2-induced facilitation on presynaptic neurotransmitter release. Surprisingly, H2O2 have no effect on neurotransmitter release from nerve terminal of a naïve motoneuron while an out-side-out patch of myocyte membrane was used as a probe to detect the ACh secretion. The Synaptic facilitation is occluded while IGF-1 antibody was added in the culture medium, suggesting IGF-1 is responsible for H2O2-induced synaptic facilitation. Overall, our current results provide evidences that H2O2 is important in developing neuromuscular synapse and further studies are needed to explore its role and underlying molecular mechanism in early synapse formation.

Disclosures: J. Liou: None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.09/G10

Topic: B.07. Synaptic Transmission

Support: Heart and Stroke Foundation of Canada

Canada Foundation for Innovation

Title: Protein phosphatase 2A activation and GluR1 AMPA receptor dephosphorylation mediate adenosine A1 receptor-mediated synaptic depression in rat hippocampus

Authors: *J. A. STOCKWELL, Z. CHEN, M. NIAZI, N. SHU, F. S. CAYABYAB;
Dept. of Physiol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Therapeutic strategies aimed at decreasing the vulnerability of neuronal tissue after stroke have been successful in animal studies, but have yet to reveal a successful therapy in humans. During ischemic stroke, extracellular levels of adenosine increase due to the extracellular breakdown of ATP as well as transport of adenosine from ischemic cells. Adenosine is an inhibitory neurotransmitter that exerts some neuroprotective effects on ischemic tissue. Previously, we identified an interaction between adenosine receptors and glutamate receptors and showed that prolonged activation of adenosine A1 receptors (A1R) mimics stroke conditions, causing the induction of persistent synaptic inhibition. Recent evidence also suggests that p38 MAPK and JNK are involved in GluR2, but not GluR1, internalization by postsynaptic A1R stimulation. We hypothesized that protein phosphatase 2A (PP2A) activation and subsequent GluR1 dephosphorylation mediates the GluR1 reduction in surface expression after stimulation with the A1R agonist CPA (500 nM). Consistent with our hypothesis, we showed that in hippocampal membrane fractions GluR1 phosphorylation at S845 and S831, two known phosphorylation sites that influence GluR1 surface expression, was significantly reduced after CPA treatment. The selective PP2A inhibitors okadaic acid (3 and 20 nM) and fostriecin (20 nM) significantly blunted CPA-induced synaptic depression using fEPSP recordings from the CA1 region of the hippocampus. Recovery of synaptic depression following CPA washout was facilitated by both PP2A inhibitors. This was supported by biotinylation studies, in which CPA significantly reduced the surface expression of both GluR1 and GluR2, and this effect was prevented by both of the PP2A inhibitors, but not by the selective protein phosphatase 1 (PP1) inhibitor tautomycetin (20 nM). Based on these data, we conclude that prolonged adenosine A1 receptor stimulation during ischemic conditions causes A1R-mediated reductions in GluR1 surface expression and accompanying persistent synaptic depression, which depend on dephosphorylation of GluR1 by PP2A activation. This newly described interaction and its role in the increased vulnerability of neurons in ischemia will be further investigated in future studies.

Disclosures: J.A. Stockwell: None. **Z. Chen:** None. **M. Niazi:** None. **N. Shu:** None. **F.S. Cayabyab:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.10/G11

Topic: B.07. Synaptic Transmission

Title: Dopaminergic agonists increase excitatory postsynaptic potentials in rat hippocampus in a region and input specific manner

Authors: *N. RIZVI, A. C. ARAI;
Pharmacol., Southern Illinois Univ. Sch. of Med., Springfield, IL

Abstract: The hippocampal-VTA dopaminergic network is implicated in motivated behavior and long term memory formation of novel and salient information. While many studies have focused on the effect of dopamine and synthetic dopaminergic agonists on CA1 pyramidal cell excitability, little attention has been given to their modulatory action in the dentate gyrus (DG). In the present study, we recorded extracellular excitatory postsynaptic potentials (EPSP) in response to perforant path (PP) stimulation in the dentate molecular layer. This was compared to recordings in CA1 stratum radiatum when Schaffer collaterals were stimulated. In both subregions, a concentration-dependent increase in EPSP amplitude was observed after application of the D1-like receptor agonist SKF38393. In the CA1 region, 20 µM drug produced a 25% increase in EPSP amplitude with no clear difference between dorsal and ventral portions of the hippocampus. In the dentate gyrus, higher concentrations were needed to produce comparable effects, but in this case the increase in EPSP amplitude showed major differences along the dorso-ventral axis and it differed between medial and lateral perforant path inputs. The largest effects of SKF38393 were observed at the medial perforant path (MPP) synapses in the dorsal DG (65%), the effect being twice as large as at ventral MPP synapses (30%). Amplitude changes at lateral perforant path (LPP) synapses were consistently smaller than those at MPP synapses. The effects of SKF38393 appeared to be irreversible and result in a long lasting increase in EPSP. In both hippocampal subregions, the SKF38393 mediated increase in EPSP amplitude was enhanced by the D1-like receptor antagonist SCH23390 and blocked by the D2 receptor antagonist sulpiride, and it was unexpectedly blocked by the phospholipase C inhibitor U73122, tentatively suggesting a role of D2 receptors in enhancing excitatory transmission through activation of Gαq signaling. Other dopaminergic agonists acting on D1-like receptors (SKF83959) and D2-like receptors (quinpirole) increased EPSP amplitude in a manner similar to

SKF38393. Our work shows that modulation of excitatory synaptic transmission in DG by dopamine exhibits gradients that may be important for hippocampal function.

Disclosures: N. Rizvi: None. **A.C. Arai:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Program#/Poster#: 231.11/G12

Topic: B.07. Synaptic Transmission

Support: NIH R01GM095653

NSERC A9935

Title: Anesthetic and ethanol enhancement of GABAA slow receptor-mediated synaptic inhibition

Authors: E. S. POSADAS[1], B. A. DAGNE[1], M. K. SUNAY[1], J. R. TRUDELL[1], B. H. BLAND[2], *B. MACIVER[3];
[1]Anesthesia, Stanford Univ., Stanford, CA; [2]Psychology, Univ. of Calgary, Calgary, AB, Canada; [3]Anesthesia, STANFORD, STANFORD, CA

Abstract: Despite widespread use and extensive research into the effects produced by anesthetics and ethanol, their mechanism(s) of action in the brain have yet to be elucidated. Recent research indicates that anesthetics and ethanol may act on extrasynaptic ‘delta’ subunit-containing GABAA receptors that generate ‘tonic’ chloride currents in neurons. We tested this hypothesis, and also asked if the drugs act on ‘non-tonic’ GABAA synaptic receptors, by measuring synaptic inhibition using field potential recordings from freely moving rats and from hippocampal brain slices, following institutional animal care approval. Stainless steel chronically implanted micro-electrodes were placed in Stratum Oriens of the CA1 region for in vivo recordings. Glass micro-electrodes were placed near the hippocampal CA1 cell body layer in brain slices prepared from 25 to 30 day old male rats. Population spikes were evoked by stimulating Schaffer-collateral fibers with bipolar electrodes in both preparations. Low concentrations of ethanol (1.0 g/kg IP or perfused: 0.10% = 22 mM), as well as higher concentrations, enhanced GABAA ‘slow’ receptor-mediated synaptic inhibition in hippocampus, seen as increased paired pulse inhibition of population spikes, at 100 ms, but not at earlier paired pulse time intervals. A similar effect was produced by isoflurane (0.2 to 1.5 vol % = 75 to 350 μM). This indicated selective enhancement of GABAA slow receptors by ethanol and isoflurane.

No evidence for effects on GABAA ‘tonic’ receptors was seen, but CA1 neurons only express delta subunit containing ‘tonic’ receptors at low levels. Thus, in this study we have shown an anesthetic and ethanol effect on GABA synapses at low concentrations associated with mild intoxication and at higher concentrations associated with loss of righting reflex.

Disclosures: E.S. Posadas: None. **B. MacIver:** None. **B.A. Dagne:** None. **M.K. Sunay:** None. **J.R. Trudell:** None. **B.H. Bland:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.12/G13

Topic: B.07. Synaptic Transmission

Support: Wings fo Life Foundation

Title: AAV2-agrin sciatic nerve delivery suppresses neuropathic pain induced by spinal cord injury

Authors: *J.-G. CUI[1], D. ERASSO[1], G. TENDER[2], R. LEVITT[1];
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Abstract: Following spinal cord injury (SCI), up to 85% of patients may develop neuropathic pain (NP). SCI-NP is associated with pathological alterations of structure, biochemistry and genes in the peripheral and central nervous systems and is a most difficult disease to treat. Existent pharmacological and surgical therapies are ineffective overtime and produce side-effect results. Agrin, a single-gene-encoded heparin sulfate proteoglycan that acts as a neuromuscular junction inducer, has also been identified as a pain mediator in the dorsal horn (DH) of spinal cord. Our previous results showed that agrin up-regulation by intraspinal injection of adeno-associated virus serum type 2 carried agrin (50 kDa) gene (AAV2-Ag50) suppressed neuropathic pain in rat peripheral nerve NP models. Based on our new results and published data, we hypothesize that AAV2-Ag50 injected into sciatic nerve will express Ag50 in the DRG and DH and suppress NP in a quisqualic acid- induced SCI NP rat model. All experiments met NIH guidelines and were approved by the University of Miami IACUC. Male Sprague-Dawley rats (240-250g) underwent quisqualic acid (QA) injection into lumbar dorsal spinal cord, which resulted in -SCI. A week after SCI, withdrawal threshold was decreased, measured by a series of Von Frey filaments. If the threshold is below 8 gram, this value is defined as tactile allodynia; if a withdrawal threshold is 20g or more after AAV2-Ag50 treatment, the threshold is defined as

normalized. Allodynic rats were randomly divided into groups subjected to an intraneural (left sciatic nerve) injection of AAV2-null, AAV2-Ag25, or AAV2-Ag50. AAV2-GFP intraneural injection was used as controls. The paw thresholds were assessed daily before and after AAV2-Ag sciatic nerve injection for three days, then the rats were perfused for immunohistochemistry or were performed for protein extraction and western blot analysis. The results were analyzed together. **I**ntraneural injections of AAV2-GFP resulted in good delivery and expression of GFP in the dorsal root ganglia and dorsal spinal cord. Intraneural injection of AAV2-Ag50 improved SCI-NP at 24 hrs and suppressed SCINP at 48 -72 hrs post-injection, while AAV2-Ag25 injection did not change the threshold. Our preliminary results suggest that 50 kDa agrin by intraneural AAV2 vector delivery can be expressed in the DRG and DH, thus suppresses SCI-NP in the rat SCI model. These results suggest that AAV2-Ag50 sciatic nerve delivery is a very promising approach for NP treatment.

Disclosures: J. Cui: None. **D. Erasso:** None. **G. Tender:** None. **R. Levitt:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Topic: B.07. Synaptic Transmission

Support: NIH grant T32-DC011499

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NIMGS grant GM065519

Title: Imaging synaptic zinc release in the dorsal cochlear nucleus with newly developed zinc sensors and chelators

Authors: *C. T. ANDERSON[1], R. J. RADFORD[3], U. PETER-APFEL[3], S. J. LIPPARD[3], T. TZOUNOPOULOS[2];
[1]Otolaryngology, [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]MIT, Cambridge, MA

Abstract: Vesicular zinc exists at many glutamatergic synapses in the brain. This d-block metal is loaded into presynaptic glutamatergic vesicles and is co-released with glutamate during synaptic transmission. The molecular layer of the dorsal cochlear nucleus (DCN) contains high levels of vesicular zinc. Our group has recently reported that synaptically-released zinc initiates endocannabinoid synthesis that subsequently modulates synaptic strength (Perez-Rosello et al.,

2013). However, the amount of synaptic zinc that reaches the postsynaptic membrane during synaptic stimulation remains unknown, mainly due to the lack of appropriate zinc sensors and chelators. Here, we have used new generation of fluorescent zinc sensors and chelators to examine how much, and under what conditions zinc is released in the DCN.

Disclosures: C.T. Anderson: None. **R.J. Radford:** None. **U. Peter-Apfel:** None. **S.J. Lippard:** None. **T. Tzounopoulos:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.14/G15

Topic: B.07. Synaptic Transmission

Support: Welcome trust

Lister institude of preventive medicine

Title: Evaluation of neuromuscular transmission in organophosphorus toxicity

Authors: *K. N. DISSANAYAKE[1], V. PATEL[3], J. MCARDLE[3], M. EDDLESTON[2], R. R. RIBCHESTER[1];
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Abstract: Organophosphorus (OP) toxicity through pesticide ingestion is a global health problem. The medical signs are complex, including an 'intermediate syndrome' from which some patients die due to respiratory paralysis. A hypothesized mechanism is initial excitotoxicity through inhibition of acetylcholinesterase followed by failure of neuromuscular synaptic transmission. We tested this electrophysiologically *in vitro* by measuring properties of spontaneous miniature endplate potentials (MEPPs) and evoked endplate potentials (EPPs) in isolated sciatic nerve/flexor digitorum brevis muscles from mice, bathed in HEPES-buffered mammalian physiological saline (MPS). Muscle action potentials were abolished with μ-conotoxin (2μM). First, we tested the effects of plasma taken from Göttingen minipigs instilled orally (isofluorane anaesthesia) with a formulated pesticide whose active ingredient is dimethoate dissolved in cyclohexanone. This plasma abolished evoked synaptic transmission and increased spontaneous MEPP frequency within 60-180 minutes of bath application. Plasma from minipigs instilled with dimethoate alone produced no failure of transmission but significantly increased the half decay time of EPPs (MPS,3.44 ±0.60 ms; control plasma,3.24±0.49 ms;

pesticide plasma, 4.10±2.38sec; dimethoate plasma,6.91±2.04sec; $p < 0.001$, ANOVA, n=20-30 fibres in each of 3-6 mice). However, pesticide-plasma also contained the metabolites omethoate (100µM) and cyclohexanol (5 mM). We found that bath application of omethoate alone caused a potent dose-dependent increase in EPP decay time. Cyclohexanol also increased EPP decay time but it also decreased both the excitability of axons and MEPP amplitude. In combination, omethoate and cyclohexanol produced greater disruption of neuromuscular transmission than either dimethoate or cyclohexanone, alone or in combination. Voltage-clamp recordings of endplate currents supported the EPP observations. Quantal analysis of EPP's also suggested that the metabolites reduced evoked neurotransmitter release. Together, the data indicate that neuromuscular transmission failure by pesticide-plasma cannot be explained solely by dimethoate-mediated inhibition of acetylcholinesterase. Rather, a combination of metabolic breakdown products exerts harmful presynaptic and postsynaptic effects. Blocking the metabolic breakdown of the constituents of OP pesticides could therefore be an effective strategy for treatment of OP pesticide toxicity.

Disclosures: K.N. Dissanayake: None. **V. Patel:** None. **J. McArdle:** None. **M. Eddleston:** None. **R.R. Ribchester:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Topic: B.07. Synaptic Transmission

Support: NIH Grant R01 DA017978

NIH Grant P50 MH086404

Title: Direct and heterogeneous control of striatal cholinergic neuron firing by midbrain dopamine neurons

Authors: *N. CHUHMA[1,2], S. MINGOTE[1,2], H. MOORE[1,2], S. RAYPORT[1,2];
[1]Columbia Univ., NEW YORK, NY; [2]NYS Psychiatric Inst., New york, NY

Abstract: Cholinergic interneurons (ChIs) comprise less than 1% of striatal (Str) neurons, yet they play crucial roles in normal reward-related behavior and motor control as well as the pathophysiology of major neuropsychiatric disorders. Str acetylcholine and dopamine (DA) reciprocally modulate each others' release; however, direct action-potential dependent control of ChIs by DA neurons has not been elucidated. We studied DA neuron input to ChIs in mouse

brain slices in three striatal domains, the dorsal striatum (dStr), nucleus accumbens (NAc) core (core) and NAc medial shell (m-shell), to address regional heterogeneity. To activate DA neuron terminals selectively, we injected a conditional channelrhodopsin 2 adeno-associated virus into the ventral midbrain of DAT-IRES-cre mice. Responses of ChIs to photostimulation of DA neuron terminals were recorded with whole cell current clamp. Short illumination (5 msec) with blue light (470 nm) evoked an IPSP in ChIs in the dStr, little response in the core, and a strong EPSP, capable of driving action potential firing in the m-shell. When DA neuron bursting was mimicked by train photostimulation (5 pulses at 20 Hz), ChIs in the dStr were hyperpolarized and paused their firing; this was mediated solely by D2 receptors. In the NAc core, train photostimulation reduced firing but did not reliably pause firing; this was mediated by GABAA and D2 receptors. In the NAc m-shell, train photostimulation caused ChIs to fire in a burst followed by hyperpolarization; the burst was glutamate mediated, while the post-burst hyperpolarization was mediated mainly by Ca2+ dependent K+ channels, and also partly by D2 and muscarinic acetylcholine receptors. Conditional knockout of vesicular glutamate transporter 2 (VGLUT2) from DA neurons eliminated the excitation, so that the response of ChIs became uniformly inhibitory across the Str domains, suggesting the regional heterogeneity was mainly due to DA neuron glutamate cotransmission. To examine plasticity in the regional heterogeneity, we treated mice with low dose (2 mg/kg) or high dose (16 mg/kg) amphetamine (AMPH), and 2.5 hours later recorded the responses of ChIs to DA neuron terminal photostimulation. Low-dose AMPH attenuated bursts in m-shell ChIs, without affecting responses in the core or dStr. High dose AMPH attenuated both the pause in the dStr and bursting in the m-shell. Thus, DA neurons transmit a fast signal to ChIs with regional heterogeneity due to blending of the synaptic actions of DA and glutamate release. The dramatic amphetamine-induced alteration in DA neuron control of ChIs is likely to figure prominently in the initial response to psychostimulants.

Disclosures: N. Chuhma: None. **S. MIngote:** None. **H. Moore:** None. **S. Rayport:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Program#/Poster#: 231.16/G17

Topic: B.07. Synaptic Transmission

Title: Measuring bioenergetic function at the nerve terminal

Authors: *D. PATHAK[1], L. SHIELDS[1], H. KIM[1], R. EDWARDS[2], K. NAKAMURA[1,2]; [1]The J. David Gladstone Inst., San Francisco, CA; [2]Dept. of Neurology, UCSF, San Francisco, CA

Abstract: Synaptic mitochondria are thought to have a critical role in supporting energy requirements at the synapse, and bioenergetic failure at the synapse may impair neural transmission and contribute to neurodegeneration. However, energy levels at the synapse have not been directly measured, due to a lack of tools to study bioenergetic function at the nerve terminal. To address this, we designed a functional assay for mitochondria-derived ATP at the synapse. The assay is based on the extent of synaptic transmission after electrical stimulation in the presence of glucose or pyruvate, and uses vesicular glutamate transporter 1 (VGLUT1)-pHluorin as a probe to monitor synaptic transmission on a bouton-by-bouton basis. We found that basal synaptic transmission can be maintained by either aerobic or anaerobic respiration. However, inhibitors of oxidative phosphorylation blocked endocytosis when glucose was absent, indicating that mitochondria-derived ATP is required for endocytosis. Further, by segregating those synaptic boutons with and without mitochondria, we showed that functionally sufficient levels of ATP diffuse into those boutons without mitochondria. To define the threshold levels of ATP required to sustain endocytosis, we also applied recently described ATP FRET sensors to dynamically monitor ATP levels at the synapse. These assays provide some of the first measurements of mitochondrial-derived energy at the nerve terminal and should be valuable tools in the assessment of energy metabolism at the synapse in health and disease.

Disclosures: D. Pathak: None. **L. Shields:** None. **H. Kim:** None. **K. Nakamura:** None. **R. Edwards:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Topic: B.07. Synaptic Transmission

Support: NS036812

Title: Developmental and NMDA receptor-dependent mechanisms contribute to changes in expression of prostaglandin E synthase isozymes in cultures of cortical neurons

Authors: Y. GONG[1], W. WANG[1], *J. A. HEWETT[2];
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Abstract: Prostaglandin E_2 (PGE_2) is a potent prostanoid metabolite of the cyclooxygenase (COX) pathway of arachidonic acid. It is generated by neurons in response to excitatory neuronal activity and, as such, is considered to be an important neuromodulator in the brain. PGE_2 is produced from free arachidonic acid by the sequential actions of COX and prostaglandin E

synthase (PGES). Two COX and three PGES isoforms have been identified. In general, expression of COX-2 and PGES-1 is inducible, whereas COX-1 and PGES-3 are constitutively expressed. Moreover, the respective isoforms couple preferentially with each other in the production of PGE_2. In contrast, PGES-2 appears to couple with either COX isoform. The specific isoforms of COX and PGES that contribute to the activity-dependent production of PGE_2 in the brain remain unknown. The purpose of this study was to assess the possible coupling of PGES-1 with COX-2 in cultures of pure cortical neurons derived from embryonic day 15 mice. Functional NMDA receptors develop after 4 days *in vitro* (DIV). COX-2 mRNA expression, as measured by quantitative PCR analysis, increased after 4 DIV as it was completely blocked by addition of the NMDA receptor antagonist, APV (30μM), on DIV 5. These results are consistent with a primary role of COX-2 in the production of PGE_2 associated with excitatory neuronal activity. Since COX-2 is coupled to PGES-1 preferentially, it is hypothesized herein that PGES-1 mRNA expression will be regulated by NMDA receptor in parallel with COX-2. Interestingly, in contrast to COX-2 expression, preliminary results suggest that PGES-1 mRNA expression was highest on DIV 1 and decreased with time in culture. Moreover, the residual expression on DIV 7 did not appear to be affected by APV treatment. Unexpectedly, however, PGES-2 mRNA expression gradually increased over 9 DIV and this was attenuated but not completely blocked by APV. These results suggest that mRNAs for PGES-1 and -2 change inversely as cortical neurons develop in culture and that the latter may be at least partly regulated by NMDA receptor activity. To date, the role of PGES-2 in production of PGE_2 in the brain has not been clearly defined. The coordinated regulation of COX-2 and PGES-2 expression raises the possibility that the production of brain PGE_2 associated with excitatory neuronal activity may be the result of specific coupling of these isoforms in neurons.

Disclosures: Y. Gong: None. **W. Wang:** None. **J.A. Hewett:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Topic: B.07. Synaptic Transmission

Support: NSERC

FRQS

Title: Dopamine D_1-family receptors enhance excitatory synaptic currents in layer II of the entorhinal cortex via activation of cAMP-PKA, PP-1, and calcium influx

Authors: *I. GLOVACI, C. A. CHAPMAN;
Concordia Univ., Montreal, QC, Canada

Abstract: The entorhinal cortex receives dopaminergic inputs that can modulate its responsiveness to synaptic inputs from other cortical areas. We have previously shown that dopamine produces reversible concentration-dependent changes in synaptic transmission in neurons in layer II of the rat lateral entorhinal cortex, such that evoked EPSPs are suppressed by high concentrations of dopamine, and facilitated by lower concentrations of dopamine. Here, we have used voltage-clamp recordings in layer II entorhinal neurons to investigate the intracellular mechanisms that mediate the synaptic facilitation effect. Results show that dopamine (1 μM), acting via D_1-family receptors, enhances pharmacologically isolated AMPA, but not NMDA, glutamate receptor-mediated currents. Elevations in PKA resulting from D_1 receptor-mediated increases in cAMP could lead to direct phosphorylation of AMPA-receptors, and might also enhance synaptic responses by increasing activity of inhibitors of protein phosphatase 1 (PP-1). Here, we found that the synaptic facilitation was blocked by intracellular application of the PKA inhibitor H-89, and that the facilitation was also blocked by the PP-1 inhibitor okadaic acid, suggesting that dopamine may facilitate AMPA-mediated responses by an inhibition of PP-1 activity. In addition, we found that intracellular application of the Ca^{2+} chelator BAPTA completely blocked the dopamine-induced facilitation of EPSCs, indicating that the facilitation of EPSCs is dependent on elevated intracellular calcium. The Ca^{2+}/calmodulin-dependent protein kinase CaMKII can enhance synaptic transmission, but we found that intracellular application of the CaMKII inhibitor KN-93 failed to reliably block the facilitation, suggesting that Ca^{2+}-induced changes in CaMKII activity are not required for the synaptic facilitation effect. The present results therefore suggest that the dopamine-induced facilitation of AMPA receptor-mediated synaptic responses in layer II entorhinal neurons is mediated via a D_1 receptor-dependent activation of the cAMP-PKA pathway, inhibition of the activity of PP-1, and elevated intracellular calcium.

Disclosures: I. Glovaci: None. **C.A. Chapman:** None.

Poster

231. **Synaptic Transmission: Modulation II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.19/G20

Topic: B.07. Synaptic Transmission

Support: RFBR Grant 11-04-00397

Russian Federation Ministry of Education and Science (Contract 8476 to Sechenov IEPhB RAS)

Title: Features of synaptic activity in primary culture of rat cortical neurons

Authors: *D. A. SIBAROV[1,2], S. M. ANTONOV[1,2];
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[2]Lab. of Mol. Neurodegeneration, Saint-Petersburg State Polytechnical Univ., Saint-Petersburg, Russian Federation

Abstract: Primary cultures of neurons from different brain regions are popular models to study neuronal physiology. Numerous studies were performed in primary cultures of neurons devoted to cellular network activity, differentiation, protein expression, intracellular calcium dynamics etc. The functional characteristics of neuronal activity in primary culture are similar to those in whole brain or in brain slices. However, some features of cultured neuron homeostasis like flat topology, deficit of glial cells and time-dependent development of synaptic functions may alter synaptic transmission. Whereas synaptic currents were widely studied in hippocampal and cerebellar cultures, few publications describing only frequencies or synaptic plasticity in primary culture of cortical neurons are available. Here we study the features of postsynaptic currents in primary culture of cortical neurons at 7 - 20 days in vitro (DIV). The use of selective blockers of ligand-gated postsynaptic ion channels in these neurons after 10 DIV revealed all types of electrical activity found in adult cortex including miniature inhibitory (mIPSCs), excitatory (mEPSCs) and spontaneous giant excitatory currents and spikes. The frequency of mEPSCs increased exponentially from 7 to 20 DIV doubling every 2.2 days in parallel with changes in action potentials generation. The mEPSCs generated by NMDA and AMPA or by only AMPA receptor activation were recorded in magnesium-free solution exhibiting postsynaptic zones of different receptor composition. In brain slices NMDA-receptors does not contribute to mEPSC amplitude in Mg-free solution. In contract to brain slices, the inhibition of presynaptic NMDA receptors by magnesium ions or AP5 influence mEPSCs amplitude by modulating their quantal content. Found discrepancies in the mechanisms of mEPSCs generation are probably due to the lack of glial control of synaptic environment.

Disclosures: D.A. Sibarov: None. **S.M. Antonov:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 231.20/G21

Topic: B.07. Synaptic Transmission

Support: NIH Grant NS073935

Title: Nerve injury increases GluR2-lacking ampa receptor prevalence in spinal cords

Authors: *S.-R. CHEN[1], H.-Y. ZHOU[2], H. BYUN[2], H.-L. PAN[2];
[1]Anesthesiol. & Perioperative Med., Univ. of Texas MD Anderson Cancer Ctr., HOUSTON, TX;
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Abstract: The glutamate AMPA receptors (AMPARs) are critically involved in the excitatory synaptic transmission, and blocking AMPARs at the spinal level reverses neuropathic pain. However, little is known about changes in the composition of synaptic AMPARs in the spinal dorsal horn after peripheral nerve injury. AMPARs lacking GluR2 subunit are permeable to Ca2+ and their currents show unique inward rectification. We found that AMPAR-mediated excitatory postsynaptic currents (AMPAR-EPSCs) of spinal dorsal horn neurons exhibited a linear current-voltage relationship in control rats, whereas AMPAR-EPSCs of dorsal horn neurons displayed inward rectification in rats with spinal nerve injury. In nerve-injured rats, compared with control rats, the GluR2 protein level was significantly less in the plasma membrane but was greater in the cytosolic vesicle fraction in the dorsal spinal cord. However, the GluR1 protein levels in these fractions did not differ significantly between nerve-injured and control rats. Blocking NMDA receptors abolished inward rectification of AMPAR-EPSCs of dorsal horn neurons in nerve-injured rats. Furthermore, inhibition of calpain or calcineurin, but not protein kinase C, completely blocked nerve injury-induced inward rectification of AMPAR-EPSCs of dorsal horn neurons. In addition, blocking GluR2-lacking AMPARs at the spinal cord level reduced nerve injury-induced pain hypersensitivity. Our study suggests that nerve injury increases GluR2 internalization and the prevalence of GluR2-lacking AMPARs in the spinal dorsal horn to maintain chronic neuropathic pain. Increased prevalence of spinal GluR2-lacking AMPARs in neuropathic pain is mediated by NMDA receptor activation and calpain-calcineurin signaling.

Disclosures: S. Chen: None. **H. Zhou:** None. **H. Byun:** None. **H. Pan:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Topic: B.07. Synaptic Transmission

Support: NIDA/NIH grant DA10044

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Title: ARPP-16, protein kinase A and MAST3 kinase: A newly identified pathway for regulation of protein phosphatase PP2A in striatal neurons

Authors: *V. MUSANTE[1], E. ANDRADE[3], J. CANIO[2], P. GREENGARD[4], A. C. NAIRN[1,4]; [1]Dept. of Psychiatry, [2]W.M. Keck Foundation,Keck MS and Proteomics, Yale Univ. Sch. of Med., New Haven, CT; [3]Lab. of molecular biology, [4]Lab. of molecular and cellular neuroscience, Rockefeller Univ., New york, NY

Abstract: Protein phosphorylation is the most common post-translational modification in neuronal signaling and results from the balanced action of kinases and phosphatases.
We previously identified a family of striatal-enriched phospho-proteins substrates for PKA: DARPP-32, RCS and ARPP-16 (Walaas et al.,1983).
While the role of DARPP-32 and RCS in the dopamine (DA)-mediated regulation of serine/threonine phosphatases in striatal medium spiny neurons (MSNs) has been well characterized (Walaas et al. 2011) little has been known about the role of ARPP-16.
ARPP-16 is related to two other members of ARPP family, ARPP-19 and ENSA. ARPP-19 and ENSA are ubiquitously distributed and have been identified as phosphatase PP2A inhibitors in mitotic cells. In Xenopus oocytes, phosphorylated by Greatwall kinase (GWT), ARPP-19/ENSA inhibit PP2A during the G2/M phase (Lorca&Castro 2013).
Recently, we found ARPP-16 directly interacts with PP2A in striatum and it is phosphorylated at Ser46 by microtubule-associated serine/threonine kinase 3 (MAST3), a mammalian analogous of GWT, enriched in striatum and still poorly characterized.
Phosphorylation of ARPP-16 at Ser46 by MAST3 converts the protein into an inhibitor of PP2A towards selective substrates including DARPP-32. Moreover, Ser46 of ARPP-16 is phosphorylated to a high basal stoichiometry in striatum, while activation of PKA by cAMP leads to marked dephosphorylation of Ser46 in striatal slices.
In the current study we further investigate the role of the phosphorylation mechanisms in ARPP-16 regulation. We demonstrated, both in vitro and in intact cells, that PKA plays a fundamental role in the MAST-mediated phosphorylation of ARPP-16 and regulation of PP2A. PKA phosphorylation of ARPP-16 at Ser88 negatively acts in an intramolecular fashion on Ser46 phosphorylation, leading to an inhibition of its ability to regulate PP2A. The phosphomimetic S88D-ARPP16 strongly suppresses MAST3 phosphorylation at Ser46 while the single PKA phosphorylation of Ser88 does not have any effect on the phosphatase inhibition. We also find that PKA phosphorylates MAST3 in vitro resulting in kinase inhibition, and that activation of cAMP by forskolin in transfected HEK cells significantly decreases MAST3 activity.
Overall these data suggest that P-Ser46-ARPP-16 acts to basally control PP2A in MSNs, but that DA, acting via PKA, regulates this pathway by inactivating ARPP-16 and/or MAST3 leading to selective potentiation of PP2A signaling.

Further experiments are in progress to identify other specific MAST3 substrates in order to characterize and better understand the role of this kinase in the regulation of the synaptic activity in striatum

Disclosures: V. Musante: None. **E. Andrade:** None. **J. Canio:** None. **P. Greengard:** None. **A.C. Nairn:** None.

Poster

231. **Synaptic Transmission: Modulation II**

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Topic: B.07. Synaptic Transmission

Support: FNRS Grant 31003A-130821/1

Biaggi Foundation

Panacée Foundation

Title: Lactate modulates neuronal excitability through both NMDA and KATP receptors: role in plasticity genes expression

Authors: *P. J. MAGISTRETTI[1,2], P. JOURDAIN[1,2], J. YANG[1], G. GRENNINGLOH[1], E. RUCHTI[1], J.-M. PETIT[1,2], I. ALLAMAN[1];
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Abstract: Release of glycogen-derived L-lactate from astrocytes has been shown to play an important role in the establishment of long term memory (Suzuki et al, Cell, 2011). In an accompanying poster, we present data demonstrating that in cultured neurons L-lactate, but not L-pyruvate, induces plasticity-related genes expression through a NMDA-dependent mechanism (i.e. Arc, Zif268 and c-Fos). Here, we describe the effect of L-lactate on neuronal excitability as revealed by electrophysiological recordings of cultured cortical neurons. We observed that application of L-lactate (10 mM) triggers an inward current with an amplitude of -0.55 +/- 0.1 nA and slow kinetics, with a peak reached at 189 +/- 55s (called Ilac). The membrane current decreases gradually to reach a plateau, with a mean inward current of -0.13 +/- 0.04 nA persisting up to 780s (13 minutes) after addition of L-lactate (called Ipyr). In order to assess L-lactate specificity in the generation of these currents, L-pyruvate as well as D-lactate, the non-metabolized enantiomer of L-lactate, were tested. In contrast to L-lactate, L-pyruvate (10 mM)

induced only a low-amplitude sustained current (-0.07 +/- 0.03 nA) similar to the late-phase slow current evoked by L-lactate (i.e Ipyr). D-lactate did not elicit any specific current. Pharmacological characterization of Ilac and Ipyr currents demonstrate that Ilac is generated by NMDA receptors (as it is blocked by MK801) and relies on active NMDA receptors (as it is blocked by either glutamate or glycine binding sites antagonists). In contrast, generation of Ipyr by both L-lactate and L-pyruvate is prevented by diazoxide, a KATP opener. In conrast Ilac was unaffected by diazoxide treatment. In order to determine the involvement of KATP for plasticity-related gene expression, the KATP closer glibenclamide (200 uM) was applied to neurons and mRNA gene expression of Arc and Zif268 determined. Results obtained demonstrate that Arc and Zif268 mRNA expression are unaffected by glibenclamide. As a whole this set of data demonstrates that L-lactate generates two types of inward currents in neurons i.e. NMDA-dependent (Ilac) or KATP-dependent (Ipyr). Moreover, they identify the NMDA-dependent Ilac current as the key mediator, in contrast to KATP, for L-lactate-induced plasticity gene expression. This set of results provides novel insights into the mechanisms of action of L-lactate in inducing plasticity gene expression, and reveals its role as a signaling molecule for neuronal plasticity.

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Poster

232. **Synaptic Transmission: Modulation III**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 232.01/G24

Topic: B.07. Synaptic Transmission

Support: M-1018-11-03-A

Title: Local agmatine injection increases striatum extracellular dopamine in rats

Authors: *L. R. BETANCOURT[1], M. SKIRZEWSKI[2], B. CATLOW[3], D. PAREDES[3], P. RADA[1], L. HERNANDEZ[1];
[1]Univ. Los Andes, Merida, Venezuela, Bolivarian Republic of; [2]Huang Lab: Section on Metabolic Redulation, NICHD, Bethesda, MD; [3]Circuit Activation & Neuronal Death, Lieber Inst. for brain development, Baltimore, MD

Abstract: NMDA receptor channel blockers like phencyclidine and ketamine, two psychotogenic drugs, enhance extracellular dopamine. Due to its highly positive charge, agmatine is attracted towards the cytoplasm through opened NMDA glutamate receptor. In the

process, agmatine plugs these cationic channels and behaves as a NMDA receptor blocker. We now report that agmatine applied by reverse microdialysis in the striatum increases extracellular dopamine in rats. A 10 mm long guide shaft, made of 21 gauge stainless steel tubing was inserted into the brain and aimed to the striatum (coordinates: 2.6 mm lateral to the midsagittal suture, 1.0 mm anterior to Bregma and 2.0 mm ventral to the surface of the brain). After a minimum of six days of recovery the rats were microdialyzed by inserting a microdialysis probe that protruded 4.0 mm from the tip of guide shaft. The inlet of the probe was connected to a syringe pump filled with artificial cerebrospinal fluid (ACSF). A basal sample was obtained 4 hours after the microdialysis probe was inserted. After 50 minutes we collected a microdialysis sample. Then we intercalated a 10 cm long PE-50 piece of tubing containing a 4.38 micromolar solution of agmatine sulphate in ACSF and after that we collected a second (50µl) microdialysis sample. The probe placement was verified by slices obtained on a Leica vibratome. Sections (40 µm) were taken through the brain areas of cannula placement, mounted onto slides and visualized in a microscope without stain by birefringency. The samples were analyzed by HPLC and EC detection as described elsewhere. Agmatine injection significantly increased DA: 237% and this increase was statistically significant ($p<0.02$). The three main matabolites of Dopamine i.e., Dihydroxiyphenilacetic acid (DOPAC), Homovanillic acid (HVA) and 3-Methoxytiramine (3MT) increased too ($p<0.04$, $p<0.04$ and $p<0.004$ respectively).These results show that agmatine injections enhance dopaminergic activity in the striatum. Evidence that rats injected with a high dose of agmatine developed schizophrenia suggest that agmatine enhances dopaminergic activity. The present experiments show a mechanism for the psychotogenic action of agmatine

Disclosures: L.R. Betancourt: None. **M. Skirzewski:** None. **B. Catlow:** None. **D. Paredes:** None. **P. Rada:** None. **L. Hernandez:** None.

Poster

232. **Synaptic Transmission: Modulation III**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 232.02/G25

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant MH56838

Title: Platelet activating factor modulates synaptic vesicle release

Authors: *J. W. HAMMOND[1], S.-M. LU[2], H. A. GELBARD[2];
[2]Ctr. for Neural Develop. and Dis., [1]Univ. of Rochester, Rochester, NY

Abstract: Platelet activating factor (PAF) is an inflammatory lipid messenger that has both physiologic and pathological functions in the brain. Normal synaptic activation leads to the formation of PAF in the postsynaptic neuron which acts as a retrograde messenger to enhance presynaptic glutamate release and thus facilitate long-term potentiation (LTP). Under pathological conditions such as HIV-1 associated dementia, seizures, and ischemia, PAF is produced by activated microglia and infiltrating monocytes. In these cases, PAF can promote neuronal excitotoxicity in a calcium, NMDA-receptor, and caspase dependent manner. The mechanism of how PAF enhances presynaptic glutamate release is poorly understood but is believed to be mediated by small G protein signaling downstream of the PAF receptor (PAFR). We show that the PAFR is indeed localized to presynaptic terminals. Using FM 1-43FX and synaptophyluorin we investigated the role of PAF in promoting spontaneous and evoked glutamate release via altering the probability of release and/or the number of the readily releasable pool of synaptic vesicles.

Disclosures: J.W. Hammond: None. **S. Lu:** None. **H.A. Gelbard:** None.

Poster

232. **Synaptic Transmission: Modulation III**

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Topic: B.07. Synaptic Transmission

Support: This study was supported by Research Grant Program at Escuela de Medicina, Universidad Anáhuac Mayab given to E.M.-R.

Title: Effects of modafinil on the expression of map-k in anterior hypothalamus and pedunculopontine tegmental nucleus of rats

Authors: *A. POOT-AKE[1], S. MIJANGOS-MORENO[1], A. MANJARREZ-MARTÍN[2], E. CRUZ-CORZO[2], E. PACHECO-PANTOJA[2], P. AQUINO- HERNÁNDEZ[2], E. MURILLO-RODRÍGUEZ[1];
[1]Lab. de Neurociencias Moleculares e Integrativas, [2]Escuela de Medicina, División Ciencias de la Salud, Univ. Anahuac Mayab, Merida, Yucatan, Mexico

Abstract: Narcolepsy is a disabling condition characterized by sleep attacks, cataplexy and excessive daytime sleepiness. Despite that the pharmacological treatment of this sleep disorder involves the use of modafinil (MOD), the molecular mechanisms that could be involved in the wake-inducing effects caused by this drug are unknown. In the present experiment, we described

the pharmacological properties of MOD if injected into two wake-related brain areas: Anterior hypothalamus (AH) or pedunculopontine tegmental nucleus (PPTg) on expression of mitogen-activated protein kinase (MAP-K). To achieve this, male Wistar rats were housed at constant temperature and under a controlled light-dark cycle. Food and water were provided ad libitum. Animals were anesthetized and a cannula was stereotaxically placed either into the AH or PPTg. One week after the surgery, the rats received an administration of MOD (10μg/1μL) at the beginning of lights-on period into either AH or PPTg and 1h after microinjections, animals were sacrificed by decapitation. Brain was collected and AH and PPTg were extracted for MAP-K analysis using Western Blot means. Preliminary results showed that MOD, injected in these two wake-related areas, promoted expression of MAP-K, compared to the respective control. Together, these results suggest that the wake-inducing properties of MOD could involve the activity of MAP-K. Further experiments are required to describe the intracellular pathway activated by MOD, which engages MAP-K.

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Poster

232. **Synaptic Transmission: Modulation III**

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Topic: B.07. Synaptic Transmission

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Brain & Behavior Research Foundation

International Mental Health Research Organization

Title: Spontaneous neurotransmitter release and synaptic plasticity

Authors: *E. D. NOSYREVA, L. MONTEGGIA, E. KAVALALI;
UTSW Med. Ctr., Dallas, TX

Abstract: Spontaneous neurotransmitter release is a salient feature of all presynaptic nerve terminals. Recent studies have shown that these action potential independent release events are essential regulators of synaptic homeostasis; in particular, they are involved in the maintenance

of synaptic strength in terms of both presynaptic release rate and postsynaptic sensitivity. Moreover, there is growing evidence that postsynaptic receptors and signaling elements that respond to spontaneous release events diverge from those that respond to evoked release, suggesting a spatial segregation of these two forms of neurotransmission. We have previously shown that application of NMDA receptor antagonists - ketamine(20 μM) and MK801 (10 μM) at rest potentiates synaptic responses in the CA1 regions of rat and mouse hippocampus. This potentiation requires protein synthesis, brain-derived neurotrophic factor expression, eukaryotic elongation factor-2 kinase function, and increased surface expression of AMPA receptors. The same synaptic potentiation could be elicited by depleting neurotransmitter selectively from spontaneously recycling vesicles. In recent experiments, we found that this form of synaptic potentiation does not fully occlude subsequent long-term potentiation elicited by theta burst stimulation (100 Hz theta-burst protocol: 100 Hz, four pulses per burst; 15 bursts at 200 ms intervals). In these experiments, we detected an additional ~20% increase in synaptic efficacy following ketamine mediated potentiation of responses (~30% above baseline). In this setting, theta burst stimulation alone could elicit up to ~50% potentiation above baseline. Taken together, these findings demonstrate that selective presynaptic impairment of spontaneous release, without alterations in evoked neurotransmission, is sufficient to elicit synaptic potentiation, which shows overlap with canonical long term potentiation elicited by repetitive activity.

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Poster

232. **Synaptic Transmission: Modulation III**

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Program#/Poster#: 232.05/G28

Topic: B.07. Synaptic Transmission

Title: The adenosine A2A receptor modulates synaptic transmission in layer 2/3 pyramidal cells of visual cortex

Authors: *N. M. BANNON, P. ZHANG, V. ILIN, M. CHISTYAKOVA, M. VOLGUSHEV; Dept. of Psychology, Univ. of Connecticut, Storrs, CT

Abstract: Adenosine is an endogenous neuromodulator which is wide spread in the central nervous system. Out of four types of adenosine receptors found in the brain (A1, A2A, A2B, and A3), the A1Rs and A2ARs are the most abundant and well-studied. A1Rs and A2ARs have different affinities to adenosine, are coupled to different G-proteins and their activation has opposite effects on synaptic transmission. Activation of A1Rs has a generally suppressive effect

on synaptic transmission that has been demonstrated in nearly every brain area, including the cortex, subcortical areas, and cerebellum. In contrast, activation of A2ARs has a facilitatory effect. A2ARs are most densely expressed within the striatum, but have been also found in the cortex. In synaptosomes from cortical tissue, A2ARs mediated facilitation of glutamate release (Marchi et al. 2002), and an antagonistic interaction between A1Rs and A2ARs has been demonstrated (Lopes et al. 1999). Some in vitro work has corroborated these actions of A2ARs in the hippocampus, but a demonstration of A2ARs effects on neocortical synaptic transmission is lacking. Due to the heterogeneity of A2AR distribution and its varied association with other receptors and intracellular cascades (Ciruela et al. 2011; Orrú et al. 2011), an understanding of the actions of A2ARs within the neocortex cannot be generalized from other structures.
Here we sought to characterize adenosine's effects on synaptic transmission in visual cortex. Using in vitro whole-cell recordings from layer 2/3 pyramidal neurons in slices of rat visual cortex, we measured effects of bath application of adenosine, the A1R antagonist DPCPX, and the A2AR antagonist SCH58261 on excitatory synaptic transmission. High concentrations of adenosine (100 - 150 μM) reduced the EPSP amplitude yet revealed an upper limit of this action. This may be due to saturation of A1Rs, but may also indicate an interaction between inhibitory A1Rs and facilitatory A2ARs. This latter scenario is supported by the following results. Application of the selective A2A antagonist SCH58261 on the background of high concentrations of adenosine revealed a small yet significant further decrease in the EPSP amplitude. Significant reduction of the EPSP after A2AR antagonist application was observed in 6 out of 24 inputs (25%). These results suggest that functional A2ARs are present in the neocortex where they oppose the effects of A1R activation, but are distributed sparsely and present not at every synapse. Thus, A1Rs might mediate an overall suppressive effect of adenosine, while A2ARs located at selected synapses and activated under specific conditions may fine-tune the neocortical transmission.

Disclosures: N.M. Bannon: None. **P. Zhang:** None. **V. Ilin:** None. **M. Chistyakova:** None. **M. Volgushev:** None.

Poster

232. **Synaptic Transmission: Modulation III**

Location: Halls B-H

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Program#/Poster#: 232.06/G29

Topic: B.07. Synaptic Transmission

Title: Effect of adenosine A1 and A2A receptors on inhibitory and excitatory transmission in layer 2/3 of rat visual cortex

Authors: *P. ZHANG[1], N. BANNON[2], V. ILIN[2], M. CHISTYAKOVA[2], M. VOLGUSHEV[2]; [1]Dept. of Physiol. and Neurobio., [2]Dept. of Psychology, Univ. of Connecticut, Storrs, CT

Abstract: Adenosine is an endogenous neuromodulator which is wide spread in the central nervous system. In the brain, there are four recognized adenosine receptors (A1, A2A, A2B, and A3), the A1 and A2A being the most abundant and well-studied. A1Rs and A2ARs have different affinities, are coupled to different G-proteins and their activation has opposite effects on synaptic transmission. Activation of A1Rs generally suppresses, but activation of A2ARs has facilitatory effect on synaptic transmission. As a result, the net effect of adenosine depends on its concentration, the presence A1Rs and/or A2ARs, and their distribution, which is highly heterogeneous in different brain regions. Evidence from several brain structures demonstrates that adenosine can modulate both excitatory and inhibitory synaptic transmission.
Here we asked: How does adenosine modulate excitatory and inhibitory transmission to layer 2/3 pyramidal neurons in rat visual cortex? Does it change the excitatory/inhibitory balance? Which receptors mediate adenosine effects? To address these questions, we made in vitro whole-cell recordings from layer 2/3 pyramidal neurons in slices of rat visual cortex, and measured effects of bath application of adenosine (5 μM to 100 μM) and antagonists of A1Rs (DPCPX, 50 nM) and A2ARs (SCH58261, 30 nM), on excitatory and inhibitory synaptic responses. We show that adenosine reduced both excitatory and inhibitory synaptic responses in a reversible and concentration dependent manner. However, concentration dependence of the reduction of inhibitory transmission was shifted towards higher concentrations relative to that of excitatory transmission. At concentrations up to 50 μM, excitatory transmission was suppressed stronger than inhibitory, resulting in a net shift of the balance towards inhibition. The adenosine-mediated suppression of either excitatory or inhibitory responses was abolished by 50 nM DPCPX, indicating the involvement of A1R. Application of A2AR antagonist SCH58261 (30 nM) on the background of 100 μM adenosine led to further suppression of excitatory responses in 6 out of 24 (25%) cases. However, when A1Rs were blocked with DPCPX (50 nM), neither 100 μM adenosine nor 30 nM SCH58261 had significant effect on synaptic responses. This indicates that facilitatory effects of A2ARs in the neocortex may be mediated via modulation of A1R affinity to adenosine and thus modulation of A1R-suppression, rather than via an independent pathway. These results suggest the role of adenosine in regulation of excitatory and inhibitory balance, and thus fine tuning of the operation of neuronal circuits of the neocortex.

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Poster

232. **Synaptic Transmission: Modulation III**

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Topic: B.07. Synaptic Transmission

Support: NIMH RO1 MH-049469

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Title: Picomolar concentrations of pregnenolone sulfate stimulate synaptic NMDAR-mediated CREB phosphorylation via an Erk signaling pathway

Authors: *C. C. SMITH, K. SUGUNAN, V. KUMARESAN, S. J. RUSSEK, T. T. GIBBS, D. H. FARB;
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Abstract: Preclinical results support the use of n-methyl d-aspartate receptor (NMDAR) modulators for cognition enhancement therapeutics. In this study we present the first report of a novel high affinity Ca++ and synaptic NMDAR-dependent effect of the neuroactive steroid pregnenolone sulfate (PregS), which we find effective at low picomolar concentrations to activate Erk-dependent phosphorylation of cyclic AMP response element binding protein (CREB). CREB is a transcription factor critical to the protein synthesis-dependent component of long term potentiation and important in associated behavioral measures of learning and memory. At micromolar concentrations, PregS is a subtype selective positive allosteric modulator of NMDARs at NR2A and NR2B containing receptors, and at concentrations ranging from pM - nM PregS induces NMDAR-dependent dopamine release in the striatum (Sadri-Vakili et al. (2008) 327: 840) and from striatal synaptosomes (Whitaker et al. (2008) 107:510). We previously demonstrated that PregS increases NMDAR surface expression in oocytes with higher potency than rapid (msec) positive allosteric modulation of NMDAR transmission. Moreover, PregS stimulated upregulation of surface NR1 subunits in cortical neurons is dependent on NMDARs but independent of channel activity. We report here that PregS increases intracellular Ca++ release in cortical neurons in a voltage-gated Na+ channel and NMDAR-NR2B dependent manner with an EC50 of ~2 pM, at least 6 orders of magnitude higher affinity than its rapid potentiating effect upon the NMDAR mediated ionotropic response, and within the range of PregS detected in bulk brain tissue. Increased intracellular Ca++ is known to induce CREB activation and we show that 50 pM PregS induces a 44 ± 13% increase in the ratio of pCREB to total CREB that is dependent upon ERK signaling and canonical excitatory synaptic transmission: this includes voltage gated Na+ channels, NMDARs, and voltage gated Ca++ channel activation. Steroid activity requires a C-3 negative charge and a saturated B-ring. Additionally, we find that 50 pM PregS increases pCREB by 65 ± 26% in hippocampal brain slices consistent with prediction from cell culture experiments. The results indicate that PregS may be a useful platform for development of novel high-affinity positive modulators of NMDAR-signaling as cognitive enhancers or therapeutics in the treatment of neurological

disorders such as Alzheimer's disease, Parkinson's disease and schizophrenia with symptoms associated with cognitive deficits.

Disclosures: C.C. Smith: None. **K. Sugunan:** None. **V. Kumaresan:** None. **S.J. Russek:** None. **T.T. Gibbs:** None. **D.H. Farb:** None.

Poster

232. **Synaptic Transmission: Modulation III**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 232.08/G31

Topic: B.07. Synaptic Transmission

Support: Parkinson's Society of Canada

Mind Foundation of BC

Title: NMDA receptor currents in pyramidal neurons of the orbitofrontal cortex are potentiated through activation of a PLC-coupled dopamine receptor pathway in juvenile but not adult rats

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Abstract: The orbitofrontal cortex (OFC) is important for decision-making about outcome expectancies of rewarding events. Alterations in excitatory synaptic transmission, including activation of NMDA receptors (NMDARs), may underlie neural processing required for decision making. Dopamine neurons project to the OFC where dopamine acts on D1-like or D2-like G protein-coupled receptors to influence decision-making. It is unknown how dopamine receptor activation modulates NMDAR EPSCs in OFC pyramidal neurons in adult and juvenile rats. We determined the effects of D1 or D2 receptor agonists on NMDAR currents in OFC pyramidal neurons from sagittal brain slices extracted from juvenile or adult male wistar rats. SKF38393, a D1-like receptor agonist, selectively potentiated NMDAR excitatory post synaptic currents (EPSCs) via a postsynaptic mechanism requiring protein kinase A in lateral OFC neurons, but not in ventral or medial OFC of adult or young rats. In contrast, quinpirole, a D2R agonist, inhibited NMDAR currents of all three OFC subregions in young or adult rats. Interestingly, co-activation of D1 and D2Rs synergistically potentiated NMDAR EPSCs significantly more than D1R activation alone in juvenile but not adult rats. This effect was attenuated by chelating intracellular calcium with BAPTA or blocking phospholipase C signaling. The synergistic effect of D1 and D2R co-activation suggested a switch in G-protein coupling through actions at D1:D2

heterodimers. Consistent with this, we found that SKF83959, an agonist which selectively activates D1:D2 dimers by the agonist potentiated currents through NMDARs in juvenile but not adult rats. These data indicate a novel mechanism by which NMDAR currents are differentially potentiated in juvenile rats versus adult rats. Furthermore, these findings provide further insight into to how dopamine modulates synaptic transmission onto OFC neurons of both adult and young rats, and may be underlie cognitive processing such as decision-making about rewards.

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Poster

232. **Synaptic Transmission: Modulation III**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 232.09/G32

Topic: B.07. Synaptic Transmission

Title: Pharmacological characterization of TMS-evoked EEG responses by positive modulators at the GABA-A receptor

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Abstract: Simultaneous registration of electroencephalography (EEG) during transcranial magnetic stimulation (TMS) enables a direct investigation of TMS-evoked cortical responses in humans at high temporal precision. Focal TMS over the primary motor cortex of one hemisphere evokes a sequence of positive and negative EEG deflections across both hemispheres lasting for about 300ms (TMS-evoked potentials, TEPs). As EEG can detect fast inhibitory postsynaptic potentials, which are linked to GABA-A receptor activity, it has been suggested that early TEPs (<50ms) are mediated, at least in part, by GABA-A receptor activity. However, the exact physiological mechanisms underlying TEPs have not been studied yet.
In this study we combined TMS-EEG with pharmacological modulation of GABA-A receptor activity to characterize the neurotransmitter systems involved in the generation of TEPs. In healthy volunteers, we conducted a pseudo-randomized, placebo-controlled, double-blind crossover study using a single oral dose of alprazolam (ALP), a classical benzodiazepine preferentially binding to alpha1, alpha2, alpha3 and alpha5 subunits of the GABA-A receptor,

and zolpidem (ZOL), which mainly binds to the alpha1-subunit. TEPs were recorded before and 90 minutes after drug administration. Drug effects on TEP amplitudes and functional cortico-cortical connectivity patterns at the sensor level using mutual information analysis were investigated. Results showed that both ALP and ZOL increased the amplitude of the negative potential at around 45 ms after stimulation (N45) to the same extent. In contrast, ALP, but not ZOL, decreased the amplitude of the negative potential at around 100 ms (N100). In addition, ALP reduced intrahemispheric connectivity contralateral to the stimulation site during early time windows after stimulation (< 50 ms), as well as long-range interhemispheric connectivity at later time windows (> 100 ms). ZOL, in contrast, decreased the connectivity between contralateral channels in the late time window only. Our results provide evidence that the N45 potential reflects activity of alpha1-subunit containing GABA-A receptors. In contrast, the breakdown of interhemispheric functional connectivity after ALP, but not ZOL, may be due to activity of non-alpha1-subunit containing GABA-A receptors.

Disclosures: I. Premoli: None. **N. Castellanos:** None. **R. Bajo:** None. **D. Rivolta:** None. **F. Muller-Dahlhaus:** None. **C. Zipser:** None. **T. Heidegger:** None. **U. Ziemann:** None.

Poster

232. **Synaptic Transmission: Modulation III**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 232.10/G33

Topic: B.07. Synaptic Transmission

Support: National Research Foundation of Korea Grant No. 2011-0005481

Title: Effects of mood stabilizers on synaptic protein levels and dendritic outgrowth in hippocampal neuronal cultures

Authors: M. SEO[1], C. LEE[1], H. CHO[1], J. LEE[1,2], B. LEE[2], W. SEOL[3], Y. KIM[1,2], *S. PARK[1]; [1]Paik Inst. for Clin. Res., [2]Dept. of Psychiatry, Sch. of Medicine, Haeundae Paik Hosp., Inje Univ., Busan, Korea, Republic of; [3]Wonkwang Univ., Gunpo, Korea, Republic of

Abstract: Purpose: Bipolar disorder is a devastating illness that is characterized by cycling periods of mania and depression. Conversing lines of evidences indicate that mood disorders, including bipolar disorder, affect abnormalities in signaling cascade mechanisms that lead to impairments in structural and functional synaptic plasticity as well as alterations in glutamatergic neurotransmission. In this study, we investigated whether mood stabilizers (lithium, valproic acid, carbamazepine, and lamotrigine) altered the expression of synaptic proteins and dendritic outgrowth in rat hippocampal neurons.

Methods: The expression of synaptic proteins, postsynaptic density protein-95 (PSD-95), brain-derived neurotrophic factor (BDNF), neurolignin 1 (NLG1), β-neurexin, and synaptophysin (SYP) in rat hippocampal neuronal cultures under toxic conditions induced by B27 deprivation was evaluated by Western blot. Additionally, dendritic outgrowth was examined to determine whether these drugs affect the dendritic morphology of hippocampal neurons.
Results: Lithium, valproic acid, and carbamazepine, significantly prevented B27 deprivation-induced decrease in levels of PSD-95, BDNF, NLG1, β-neurexin and SYP ($p < 0.05$ or $p < 0.01$), whereas lamotrigine had no effect in this regard. All the drugs significantly increased the total outgrowth of hippocampal dendrites ($p < 0.01$). Moreover, this effect of lithium and valproic acid is blocked by specific inhibitors of calcium/ calmodulin kinase II (CaMKII), KN-93 (1 μM), protein kinase A (PKA), H-89 (1 μM), phosphatidylinositol 3-kinase (PI3K), LY294002 (10 μM), or MAPK/ERK kinase (MEK), PD98059 (1 μM) signaling ($p < 0.05$ or $p < 0.01$), whereas other mood stabilizers do not affect these signaling.
Conclusions: Taken together, these results suggest that certain mood stabilizers may regulate synaptic plasticity by enhancing synaptic protein levels and dendritic outgrowth in hippocampal neurons. In addition, these effects on dendritic outgrowth likely require CaMKII, PKA, PI3K or MEK signaling pathways.
Key words: Mood stabilizers; Hippocampal plasticity; Synaptic proteins; Dendritic outgrowth; Signaling

Disclosures: M. Seo: None. **S. Park:** None. **C. Lee:** None. **H. Cho:** None. **J. Lee:** None. **B. Lee:** None. **W. Seol:** None. **Y. Kim:** None.

Poster

232. **Synaptic Transmission: Modulation III**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 232.11/G34

Topic: B.07. Synaptic Transmission

Title: Fluoxetine impairs GABAergic signaling in hippocampal slices from neonatal rats

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Abstract: Fluoxetine (Prozac), an antidepressant known to selectively inhibit serotonin reuptake, is widely used to treat depression during pregnancy and postpartum period. Several lines of evidence suggest that this drug exerts its action not only by interfering with serotoninergic but also with GABAergic transmission which, early in postnatal life, plays a crucial role in the

construction of neuronal circuits.
Here we tested the hypothesis that fluoxetine may interfere with GABAergic signaling at early developmental stages, thus producing harmful effects on brain development.
Correlated network activity such as GDPs, a hallmark of developmental circuits, were recorded in whole cell configuration of the patch clamp technique in acute hippocampal slices from P2-P6 old rats. Bath application of fluoxetine (20 μM) reversibly reduced GDPs frequency (from 0.08 ± 0.04 Hz to 0.045 ± 0.031 Hz; n=5; p = 0.021). This effect was concentration dependent (IC_{50} value: 22 μM) and independent of serotonin action since it persisted in the presence of citalopram, a selective serotonin uptake inhibitor and after pharmacological occlusion of monoamine receptors with asenapine.
We next examined whether fluoxetine-induced changes in spontaneous action potential dependent and independent GABA and glutamate release. The drug reduced the frequency (from 4.7 ± 0.9 Hz to 3.3 ± 0.76 Hz; n = 8; p = 0.007) and the amplitude (57 ± 7 pA to 47 ± 8 pA; n = 8; p = 0.004) of spontaneous $GABA_A$-mediated postsynaptic currents without altering spontaneous AMPA-mediated excitatory postsynaptic currents, indicating that the reduction in GDPs frequency probably involves GABAergic but not glutamatergic signaling.
To evaluate whether fluoxetine selectively affects a particular interneuron population we used WIN55,212-2, a CB1/CB2 receptor agonist, to occlude cannabinoid receptors present on axon terminals of cholecystokinin-positive (CCK) regular spiking cells. In the presence of WIN55,212-2 (1μM), fluoxetine was still effective, indicating that CCK-positive GABAergic interneurons were not involved.
In addition, fluoxetine reduced the firing rate of both principal cells (from 1.57 ± 0.74 Hz to 0.56 ± 0.37 Hz; n = 5; $p < 0.05$) and interneurons (from 2.7 ± 1.7Hz to 0.33 ± 0.22 Hz; n = 5;$p < 0.05$) further suggesting that changes in network excitability account for fluoxetine-induced GDPs disruption. This may have critical consequences on the functional organization and stabilization of neuronal circuits early in postnatal development.

Disclosures: M.D. Caiati: None. **E. Cherubini:** None.

Poster

232. **Synaptic Transmission: Modulation III**

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Program#/Poster#: 232.12/G35

Topic: B.07. Synaptic Transmission

Support: INSERM

MRT

ANR

Title: Role of kainate receptors in the pathophysiology of temporal lobe epilepsy

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Abstract: Kainate (KA), an analogue of glutamate, is a potent neurotoxin known to induce seizures reminiscent of those found in patients with temporal lobe epilepsy (TLE) (Ben-Ari & Represa, 1990). Whether KA receptors (KARs)are key elements in the triggering and propagation of seizure discharges is yet unclear. In both human patients and animal models of TLE, neuronal tissue undergoes major reorganization; some neurons die while others sprout and form novel aberrant connections (Represa, et al. 1989; Nadler, 2003; Blaabjerg & Zimmer, 2007; Dudek & Sutula, 2007; Ben-Ari et al., 2008). This phenomenon is best documented in the dentate gyrus where mossy fiber (MF) axons sprout to form aberrant glutamatergic excitatory synapses onto other dentate granule cells (DGCs) leading to the formation of functional recurrent excitatory circuits (Tauck & Nadler, 1985; Wuarin & Dudek, 1996; Molnar & Nadler, 1999; Lynch & Sutula, 2000; Buckmaster et al., 2002; Scharfman et al., 2003). This accounts for, in part, the enhanced ability of the hippocampus to generate epileptiform activity (Patrylo & Dudek, 1998; Hardison et al., 2000; Gabriel et al., 2004). MF sprouting also induces a reorganization of KAR-mediated synaptic transmission, with a shift in the nature of glutamatergic transmission in DGCs. 50% of recurrent MF inputs that impinge on DGCs operate via KARs and drive synaptic events with abnormal long lasting kinetics not present in naïve conditions (Epsztein et al., 2005, Epsztein et al., 2010). As a result, the sparse firing of DGCs is switched to an abnormal sustained and rhythmic mode (Artininan et al. 2011). The present study explores the pathophysiological implications of KARs in animal models during the chronic phase of TLE through the use of KAR-subunit deficient mice and selected pharmacological agents.

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Poster

232. **Synaptic Transmission: Modulation III**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 232.13/G36

Topic: B.07. Synaptic Transmission

Title: Potentiation of GABA-mediated synaptic inhibition as a novel target for post-stroke recovery

Authors: *T. HIU[1], T. BLISS[2], J. PAZ[3], E. WANG[2], A. OLSON[4], K. MICHEVA[5], Z. FARZAMPOUR[3], G. WANG[5], K. TRAN[2], N. MANLEY[2], Y. NISHIYAMA[2], A. ARAC[2], N. O'ROURKE[5], J. HUGUENARD[3], S. SMITH[5], G. STEINBERG[2];
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Abstract: Stroke is a major cause of severe disability, yet pharmacological therapy to promote post-stroke recovery is deficient. Understanding the stroke induced molecular and cellular alterations in the peri-infarct area, the region adjacent to the damaged stroke site, is critical to developing new therapeutics to enhance stroke recovery. Reducing excessive tonic neuronal inhibition, mediated by extrasynaptic $GABA_A$ receptors, promotes functional recovery after stroke. However, the role of the phasic (synaptic) GABA inhibition in recovery remains unknown. We previously reported a transient structural and functional increase in phasic inhibition of layer 5 pyramidal neurons in the peri-infarct area. We found, using array tomography, an increase in the number of GABAergic synapses containing the α1 receptor subunit in layer 5 of the peri-infarct cortex, but not layer 2/3, and an associated increase in spontaneous inhibitory post-synaptic currents specific to layer 5 pyramidal neurons. This effect was transient, occurring during the onset of functional recovery. To test whether the increased phasic inhibitory GABAergic signaling promotes stroke recovery, we treated animals with zolpidem, an agonist with high affinity for α1 subunit-containing $GABA_A$ receptors. We find that low dose zolpidem increases $GABA_A$ phasic signaling in layer 5 pyramidal cells and notably increases the rate and extent of behavioral recovery without altering the infarct size. These data provide the first evidence that enhanced phasic $GABA_A$-mediated synaptic activity improves recovery after stroke. Our results introduce modulation of phasic $GABA_A$-mediated synaptic inhibition as a novel therapeutic strategy, identify zolpidem as a potential pharmacological agent to improve stroke recovery, and underscore the necessity to distinguish the role of tonic inhibition (extrasynaptic) and phasic inhibition (synaptic) in stroke recovery. We propose that phasic GABA signaling is as critical for adaptive plasticity after stroke as during development.

Disclosures: T. Hiu: None. **T. Bliss:** None. **J. Paz:** None. **E. Wang:** None. **A. Olson:** None. **K. Micheva:** None. **Z. Farzampour:** None. **G. Wang:** None. **K. Tran:** None. **N. Manley:** None. **Y. Nishiyama:** None. **N. O'Rourke:** None. **J. Huguenard:** None. **S. Smith:** None. **G. Steinberg:** None. **A. Arac:** None.

Poster

232. **Synaptic Transmission: Modulation III**

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Program#/Poster#: 232.14/G37

Topic: B.07. Synaptic Transmission

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Title: JZ-V improved learning and memory impairment and accelerated perforant path-dentate gyrus LTP in intact animals

Authors: *X. WANG, X. CHEN, X. LIU, Y. XIE, T. QIAN;
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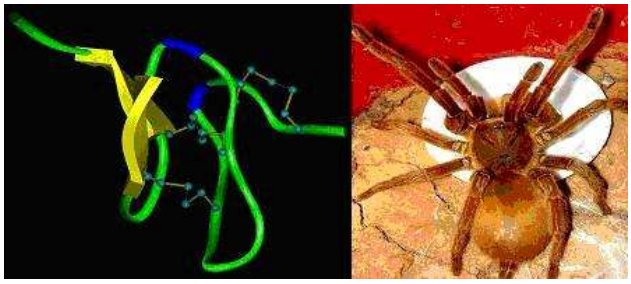
Abstract:
Aim: The purposes of this study were to investigate the effect of JZ-V, a novel peptide neurotoxin isolated from the venom of spider Chilobrachys jingzhao and composed of 29 residues including six cysteines and three exposed tryptophan residues on learning and memory and LTP. **Methods:** Learning and memory impairment was induced by beta-amyloid1-42 and Scopolamine, and the behavior was evaluated by Morris water maze and Step-through experiments to investigate the effect of JZ-V on spatial leaning and memory and passive avoidance learning and memory. In Morris water maze test, the latency, swimming distance, the searching strategy were recorded and the error numbers and the latency were recorded in Step through test. In addition, extracellular technique was used to determine the effect of JZ-V on LTP induction in medial perforant path dentate granule cell synapses, single- pulse stimulation was delivered to perforant path fibers in vivo. In slice recording system, the Schaffer-CA1 pathway LTP was recorded in 400 microm-thick transverse hippocampal slices. **Results:** JZ-V significantly enhanced LTP induction, the population spike was increased from180±11 % to about 332± 28 % of control in perforant path-dentate gyrus pathway 20 minutes after, high frequency stimulation. However, the results from slice recording was quite difference with that of anesthetized rats, the slope of EPSP was increased from about 141± 12 % to 159±14 % of control 10 minues after high frequency stimulation. From behavior results, JZ-V significantly reduced escape latency and swimming distance in place navigation test, and it significantly prolonged passive avoidance latency and decreased the error numbers during 5 minutes in step-through test. **Conclusions:** JZ-V significantly improved impaired learning and memory induced by beta-amyloid and Scopolamine, which showed some potential to be a promising new trial

drug for Alzheimer's disease. The present study gave the strong evidence that learning and memory behavior is associated with LTP, and this is the first report about JZ-V on learning and memory and LTP.



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Poster

232. **Synaptic Transmission: Modulation III**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 232.15/G38

Topic: B.07. Synaptic Transmission

Support: French Association against Myopathies

Region Alsace

Mulhouse Area m2A

Alsace Business Angels

SODIV

Title: Identification of synergistic drug combinations using modeling and simulation of Long-Term Potentiation

Authors: A. F. KELLER[1], N. AMBERT[1], A. LEGENDRE[1], R. GREGET[1], M. SARMIS[1], M. BEDEZ[1], F. LALOUE[1], J. KOENIG[2], J.-M. C. BOUTEILLER[1,3], *S. BISCHOFF[4], T. W. BERGER[1,3], M. BAUDRY[1,5];
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Abstract: Cognitive deficit is a common feature of many neurodegenerative diseases, such as Alzheimer's disease. While several drugs are currently available, their effects on cognition are relatively minimal and there is a need to identify better treatment. Long-term potentiation (LTP) of synaptic transmission has been widely used as a cellular model of learning and memory, and has been typically elicited by either a 1 second-long high frequency stimulation (HFS) at 100 Hz, or a succession of 4-6 stimuli at 100 Hz, called bursts, spaced by 200 ms (theta burst stimulation, TBS). We first used a simulation approach modeling CA1 pyramidal cells and the CA3 afferents with 30 synapses distributed on a branch of a CA1 pyramidal neuron. NMDA, AMPA, and the metabotropic type 1 glutamate receptors were included at each synapse. The LTP model was based on Shouval (2005) and links changes in intracellular calcium to the number of AMPA receptors. In vitro experiments were performed with acute rat hippocampal slices, and field potentials elicited by stimulation of the Schaffer collaterals were recorded in CA1 stratum radiatum. In vivo, a fear-conditioning paradigm was used to assess effects of drug combinations on learning and memory of adult rats. A glutamatergic drug and three cholinergic modulators were tested separately and in combination to evaluate possible synergistic effects on in silico LTP, experimental LTP and behavior. One combination was predicted to be synergistic with the simulation using the TBS paradigm but not HFS, which was due to the saturation of potentiation with HFS at each synapse. The synergic effects of this combination was verified both experimentally in vitro with TBS of hippocampal slices, and in vivo with the fear-conditioning behavior. Thus, these results demonstrated that drugs have different effects depending on the experimental protocol used, identified a specific combination with synergistic effects on LTP and on learning and memory. They also indicate that synergistic drug combinations can be discovered using first computer modeling and simulation. As drug combinations are likely to become the future for treating complex diseases such as neurodegenerative ones, the results open new avenues for improved treatments.

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Poster

233. **LTP: Kinases and Intracellular Signaling**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 233.01/G39

Topic: B.08. Synaptic Plasticity

Title: Regular exercise prevented hippocampal L-LTP impairment and aberrant alterations in levels of L-LTP related signaling molecules in Alzheimer's disease-like pathology

Authors: *A. DAO[1], M. ZAGAAR[2], S. SALIM[1], K. ALKADHI[1];
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Abstract: Alzheimer's disease (AD) is an insidious gradual deterioration of intellectual and emotional wellbeing. The disease is hallmarked by extracellular accumulation of high levels of neurotoxic amyloid-beta (Aβ) peptides, followed later by intracellular aggregation of hyperphosphorylated tau protein, leading to neuronal death and progressive loss of mental abilities. Current AD medications delay the disease symptoms for only 6 months and are effective only in half of the patients; thus, there is an urgent need for innovative therapies. Regular exercise has been shown to be beneficial for brain health. We have reported that 4 weeks of treadmill exercise prevented short-term memory impairment and suppression of early phase long-term potentiation (E-LTP) in CA1 area in a rat model of AD (Aβ1-42 peptides, 250 pmol/day, i.c.v. infusion for 2 weeks). In continuation of our previous study, we investigated whether this exercise regimen was able to prevent late phase LTP (L-LTP) inhibition in both CA1 and DG areas and the molecular pathways that could be involved in the beneficial effect of exercise in our AD model. We found that AD pathology blocked L-LTP in both CA1 and DG areas of the hippocampal formation and that this effect was prevented by 4 weeks of treadmill exercise. Additionally, treadmill exercise also prevented AD-induced reduction in the levels of signaling molecules such as phosphorylated cAMP response element-binding protein (CREB), calcium-calmodulin dependent protein kinase IV (CaMKIV) and brain derived neurotropic factor (BDNF). Together, these findings suggest a neuroprotective action of exercise against AD pathologies and possibly other neurodegenerative diseases.

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Poster

233. **LTP: Kinases and Intracellular Signaling**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 233.02/G40

Topic: B.08. Synaptic Plasticity

Support: National Institute of Health (MH64856 and NS36715)

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National Institute of Health (T32MH15330)

Deutsche Forschungsgemeinschaft

Title: Analysis of atypical PKCs in hippocampal synaptic plasticity and memory

Authors: *L. J. VOLK[1], J. L. BACHMAN[1], R. C. JOHNSON[1], Y. YU[1], X. YUE[1], M. LEITGES[2], R. L. HUGANIR[1];
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Abstract: Long-term potentiation (LTP), a well-characterized form of synaptic plasticity, has long been postulated as a cellular correlate of learning and memory. While LTP can persist for very long periods of time, the mechanisms underlying LTP maintenance, in the midst of on-going protein turnover and synaptic activity, remain elusive. Sustained activation of the brain-specific atypical protein kinase C isoform, protein kinase M zeta (PKMζ), has been reported to be necessary for both LTP maintenance and long-term memory. Inhibiting PKMζ activity using a synthetic peptide (ZIP) based on the PKCζ pseudosubstrate sequence reverses established LTP *in vitro* and *in vivo*. More strikingly, infusion of ZIP eliminates memories for an ever-growing list of experience-dependent behaviors. However, the specificity of ZIP remains controversial, and most of the evidence supporting a role for PKMζ in LTP and memory relies heavily on pharmacological inhibition of PKMζ by ZIP. In recently published data we found that both conventional and conditional PKC/Mζ knockout (KO) mice show normal synaptic transmission and LTP at Schaffer collateral-CA1 synapses. Additionally, mice lacking PKMζ showed no deficits in several hippocampal-dependent learning and memory tasks indicating that normal synaptic plasticity, learning, and memory can occur in the absence of PKMζ. Surprisingly, ZIP still reversed LTP in PKC/Mζ KO mice indicating that ZIP's effects, while remarkable, are independent of PKMζ.
We are currently conducting experiments to identify the functional target(s) of ZIP and to determine if PKCλ/ι, the other atypical PKC, can support LTP in the absence of PKMζ. *In vitro* kinase assays suggest that ZIP is a general PKC inhibitor, exhibiting no appreciable specificity across multiple isoforms. Using an alternative method to generate conditional PKC/Mζ knockout in adult mice (AAV-mediated Cre expression in the hippocampus), we have confirmed our previous results that, in the absence of potential developmental compensation, hippocampal neurons lacking PKMζ are capable of exhibiting normal long lasting LTP. Additionally, our preliminary findings suggest that LTP is also unaffected by conditional knockout of PKCλ. Future studies in double PKCζ/PKCλ knockout mice will determine if the presence of at least one atypical PKC is necessary for normal hippocampal LTP and memory.

Disclosures: L.J. Volk: None. **J.L. Bachman:** None. **R.C. Johnson:** None. **Y. Yu:** None. **X. Yue:** None. **M. Leitges:** None. **R.L. Huganir:** F. Consulting Fees (e.g., advisory boards); Millipore Corporation.

Poster

233. **LTP: Kinases and Intracellular Signaling**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 233.03/G41

Topic: B.08. Synaptic Plasticity

Support: Philip Whitcome Pre-doctoral Training Program in Molecular Biology (to MD)

R01MH077022 (to KCM)

Title: Dynamic activity-dependent dephosphorylation of CRTC1 regulates synapse to nuclear transport

Authors: *M. DESALVO, T. H. CH'NG, A. VASHISHT, J. WOHLSCHLEGEL, K. C. MARTIN;
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Abstract: CREB Regulated Transcriptional Co-activator 1 (CRTC1) undergoes activity-dependent synapse to nucleus translocation and has been shown to play a role in neuronal plasticity and memory. In non-neuronal cells, CRTC1 is sequestered in the cytoplasm by interacting with 14-3-3 proteins. Increased levels of calcium and cAMP lead to CRTC1 dephosphorylation, which releases it from 14-3-3 and allows it to enter the nucleus. In the nucleus, CRTC1 binds CREB (and other bZIP transcription factors) and recruits transcriptional machinery to robustly potentiate CRE-dependent transcription, which is crucial for induction of the late phase of long-term potentiation (L- LTP). Our lab has shown that CRTC1, at rest, interacts with 14-3-3 ε and localizes to the synapses in rodent hippocampal neurons. Glutamatergic stimulation causes dynein-dependent retrograde transport along microtubules and nuclear import of CRTC1.
We have shown that CRCT1 is heavily phosphorylated and that its phosphorylation state changes upon stimulation. Our current efforts are focused on identifying the phosphorylation sites that are important for synapse to nuclear transport of CRTC1. We hypothesize that different types of stimulation lead to distinct phosphorylation states, which in turn regulate the nucleocytoplasmic trafficking of CRTC1 as well as its transcriptional activity in the nucleus. We are in the process of identifying and characterizing phosphorylation sites that could be important for these processes in neurons. Using affinity purification and mass spectrometry, we have also identified several kinases and phosphatases that interact with CRTC1 and that may play a role in its nuclear accumulation. Understanding the role of phosphorylation in the nuclear transport of CRTC1 will give us a deeper understanding of its role in activity-dependent plasticity in neurons.

Disclosures: M. Desalvo: None. **T.H. Ch'ng:** None. **A. Vashisht:** None. **J. Wohlschlegel:** None. **K.C. Martin:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

Location: Halls B-H

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Program#/Poster#: 233.04/G42

Topic: B.08. Synaptic Plasticity

Support: Grant-in-Aid for Scientific Research for Young Researcher

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CIHR grant MOP 111220, Canada Research Chairs Program, Canadian Foundation for Innovation

Title: CaMKIIβ as a gating mechanism of activity-induced structural modification of hippocampal dendritic spines

Authors: *K. KIM[1], G. LAKHANPAL[2], A. SUZUKI[1], M. HAYASHI[3], R. NARAYANAN[4], T. MATSUDA[5], T. NAGAI[5], Y. HAYASYI[1,6,7], K. OKAMOTO[2,8];
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Abstract: The size of the synapse is dynamically regulated during synaptic plasticity, therefore, it is conceived that the mechanism regulating the size of synapse is tightly linked to the mechanism of plasticity. Here we demonstrate that Ca^{2+}/calmodulin-dependent protein kinase (CaMKII), a pivotal kinase in synaptic plasticity, mediates activity-dependent structural modification of excitatory synapses through a novel activity-regulated F-actin stabilizing function, apart from well-known kinase signaling. When CaMKII is inactive state, it bundles F-

actin but when it is activated by Ca^{2+}/calmodulin, it undergoes autophosphorylation reaction on newly identified sites within the actin-binding domain and unbundles F-actin. This mechanism opens a temporary time window of ~1 min where F-actin is remodeled by actin modulating proteins such as cofilin, Arp2/3, and gelsolin, leading to the structural modification of dendritic spines. These observations make CaMKII a unique F-actin regulatory molecule with a permissive role in gaiting activity-dependent modification of synaptic structure.

Disclosures: K. Kim: None. **G. Lakhanpal:** None. **A. Suzuki:** None. **M. Hayashi:** None. **R. Narayanan:** None. **T. Matsuda:** None. **T. Nagai:** None. **Y. Hayasyi:** None. **K. Okamoto:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

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Topic: B.08. Synaptic Plasticity

Support: SIU Startup Fund

NIMH R01

Title: Persistent activation of protein kinase A is involved in serotonin-induced long-lasting potentiation of TA-CA1 excitatory synaptic transmission in rodent model of depression

Authors: *X. QI[1,2], Z. YUAN[1], P. R. PATRYLO[1,2,3], G. M. ROSE[2,3], S. M. THOMPSON[4], X. CAI[1];
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Abstract: Depression is a leading cause of suicide and one of the top ten causes of mortality and morbidity worldwide. The biological basis of cognitive dysfunction in major depression remains unknown. Emerging evidence indicates that malfunction of excitatory synapses is involved in the pathophysiology of depression. Our previous work showed that activation 5-HT_{1B} receptors by endogenous serotonin selectively potentiated excitatory synapses formed by the temporoammonic (TA) pathway with CA1 pyramidal cells via activation of 5-HT_{1B}Rs without affecting nearby Schaffer collateral synapses. Serotonin induced a long-lasting potentiation of TA-CA1 EPSPs in animals subjected to chronic unpredictable stress (CUS), an animal model of

depression. In the current study, we compared the phosphorylation of Ser831 and Ser845 on AMPA receptor GluR1 subunit upon application of 5-HT_{1B}Rs agonist, anpirtoline, between control and CUS animals. We observed that anpirtoline increased Ser831 phosphorylation in both control and CUS animals. However, anpirtoline only enhanced phosphorylation of S845 in CUS animals; this phosphorylation remained elevated even after washout of anpirtoline. Moreover, slices from control animals pretreated with Sp-cAMPs, an activator of protein kinase A, prolonged anpirtoline-induced TA-CA1 EPSPs, which mimicked anpirtoline-induced the long-lasting potentiation in CUS animals . Our results suggest that pathological activation of PKA participates in serotonin-induced long-lasting potentiation of TA-CA1 EPSPs in brain slices from CUS animals.

Disclosures: X. Qi: None. **Z. Yuan:** None. **P.R. Patrylo:** None. **G.M. Rose:** None. **S.M. Thompson:** None. **X. Cai:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

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Program#/Poster#: 233.06/G44

Topic: B.08. Synaptic Plasticity

Support: NRF Grant 2009-0081468

NRF Grant 2011-0027667

Title: Abnormal hippocampal synaptic plasticity in c-Jun N-terminal phosphorylation mutants mice

Authors: *S.-Y. CHOI, J. SEO, J. HONG, S. LEE;
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Abstract: c-Jun N-terminal kinase (JNK), a member of the MAPK family, is an important regulatory factor of synaptic plasticity as well as neuronal differentiation and cell death. Recently, JNK has been reported to modulate synaptic plasticity by the direct phosphorylation of synaptic proteins. The specific role of c-Jun phosphorylation in JNK mediated synaptic plasticity, however, remains unclear. In this study, we investigated the effects of c-Jun phosphorylation on synaptic structure and function by using c-Jun mutant mice, c-JunAA, in which the active phosphorylation sites at serines 63 and 73 were replaced by alanines. The gross hippocampal anatomy and number of spines on hippocampal pyramidal neurons were normal in c-JunAA mice. Basal synaptic transmission, input-output ratios, and paired-pulse facilitation

(PPF) were also no different in c-JunAA compared with wild-type mice. Notably, however, the induction of long-term potentiation (LTP) at hippocampal CA3-CA1 synapses in c-JunAA mice was impaired, whereas induction of long-term depression (LTD) was normal. These data suggest that phosphorylation of the c-Jun N-terminus is required for LTP formation in the hippocampus, and may help to better characterize JNK-mediated modulation of synaptic plasticity.

Disclosures: S. Choi: None. **J. Seo:** None. **S. Lee:** None. **J. Hong:** None.

Poster

233. LTP: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: Rajiv Gandhi Centre for Biotechnology, India

Department of Biotechnology, Government of India

Department of Science and Technology, Government of India

Title: CaMKIINα and GluN2B exhibit similar modes of regulation of CaMKII function

Authors: *R. V. OMKUMAR[1], S. JOHN[2], M. MADHAVAN[2];
[1]Mol. Neurobio. Div., Mol. Neurobio. Div., Thiruvananthapuram, India; [2]Mol. Neurobio. Div., Rajiv Gandhi Ctr. for Biotech., Thiruvananthapuram, India

Abstract: Calcium/calmodulin dependent protein kinase II (CaMKII) is a Ser/Thr protein kinase with a unique multimeric structure. It supports many higher order brain functions such as learning and memory. There are multiple mechanisms for the intracellular regulation of CaMKII activity. CaMKIINα has been identified as a natural protein inhibitor of CaMKII and resembles the NMDA receptor 2B subunit (GluN2B) in its mode of interaction with CaMKII (Vest et al, 2007, Mol Biol Cell; Vol.18; p5024). This prompted us to investigate whether CaMKIINα regulates the function of CaMKII in a manner similar to GluN2B. We have used purified preparations of the proteins and *in vitro* assays for phosphorylation and dephosphorylation activities. We find that dephosphorylation of phospho-Thr^{286}-α-CaMKII by protein phosphatase 1 (PP1) is attenuated by CaMKIINα similar to the regulatory effect reported for GluN2B (Cheriyan *et al*, 2011, PLoS One; Vol. 6: e16495). This suggests that the structural changes on CaMKII brought about by the interaction of CaMKIINα and GluN2B are likely to be similar. We are currently investigating

whether there are similarities in the modulation of catalytic activity of CaMKII brought about by CaMKIINα and GluN2B.

Disclosures: R.V. Omkumar: None. **S. John:** None. **M. Madhavan:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

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Program#/Poster#: 233.08/G46

Topic: B.08. Synaptic Plasticity

Support: NIH Grant FNS079083A

Title: Mechanisms of compartmentalization of the activated cAMP dependent protein kinase

Authors: *S. E. TILLO, H. ZHONG;
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Abstract: Throughout the brain, the cAMP dependent protein kinase (PKA) plays critical roles in neurotransmission, cell excitability, and synaptic plasticity. All of these processes require high specificity in PKA phosphorylation profiles. Previous studies have shown that PKA specificity is established by a class of proteins called A-kinase anchoring proteins (AKAPs), which target upstream activators and downstream substrates of PKA to specific subcellular locations. However, AKAPs only bind the regulatory subunit of PKA. Once activated by cAMP, the catalytic subunit of PKA is released from the regulatory subunit and is free to diffuse. A cytosolic protein of comparable size to the PKA catalytic subunit will equilibrate synaptic compartments, such as dendrites and their spines, in tens of milliseconds and exit these compartments within hundreds of milliseconds. As these time scales are significantly shorter than the known time scales of PKA signaling, such diffusion would be expected to break down the PKA specificity established by AKAPs. How does PKA signaling maintain its specificity despite the freely diffusing catalytic subunit? Our results suggest that in addition to the subcellular targeting provided by AKAPs, that constrained diffusion of the PKA catalytic subunit is necessary for proper and efficient phosphorylation of downstream targets.

Disclosures: S.E. Tillo: None. **H. Zhong:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

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Topic: B.08. Synaptic Plasticity

Support: P01NS045260-01

Title: Dual and opposite functions for calpain in long-term potentiation induction and consolidation

Authors: *G. ZHU[1], Y. WANG[1], V. BRIZ[1], Y.-T. HSU[2], X. BI[3], M. BAUDRY[1];
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Abstract: Long-term potentiation (LTP) is widely recognized as a cellular model of learning and memory. While much has been learned regarding the role of various cellular mechanisms in LTP, the role of the calcium-dependent protease, calpain, has remained controversial. Here we report that calpain performs two distinct and opposite functions in LTP induction and consolidation elicited by theta burst stimulation (TBS): on the one hand, calpain activation is necessary for LTP induction, as calpain inhibition before TBS prevents LTP; on the other hand, calpain activation limits the magnitude of LTP during the consolidation period, as calpain inhibition up to 1 hour after TBS enhances LTP. Moreover, these dual functions of calpain are directly related to its sequentially opposite effects on ERK activation. Calpain rapidly activates ERK by degrading suprachiasmatic nucleus [SCN] circadian oscillatory protein (SCOP), a negative ERK regulator, during LTP induction, and restricts ERK activation by stimulating the mTOR-dependent synthesis of SCOP during LTP consolidation. Blocking local protein synthesis with rapamycin shortly after TBS also results in LTP enhancement. These results provide a new framework to understand the spatio-temporal events underlying synaptic plasticity and learning and memory, and could provide a molecular mechanism for the beneficial effects of space vs massed trial learning.

Disclosures: G. Zhu: None. **Y. Wang:** None. **V. Briz:** None. **Y. Hsu:** None. **X. Bi:** None. **M. Baudry:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

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Topic: B.08. Synaptic Plasticity

Support: CIHR IRSC

NSERC CRSNG

Title: The role of calpain-mediated cleavage of GluN2B in dendritic spine signaling and remodeling

Authors: *F. EL GAAMOUCH, K. DORE, S. LABRECQUE, M. LEMIEUX, B. TOURNIER, P. DE KONINCK;
Neurobiologie Cellulaire, CRIUSMQ, Quebec, QC, Canada

Abstract: Synaptic plasticity in the hippocampus is a prominent cellular mechanism of learning and memory. During elevated activity at excitatory synapses, the Ca2+ influx through NMDA-type glutamate receptors (NMDAR) activates a multitude of signaling processes in spines, leading to long-term potentiation (LTP) of synaptic transmission. The cytoplasmic tail of GluN2 subunits of NMDARs acts as a central hub for several critical interactions in synaptic plasticity. Previous work suggests that CaMKII interaction with GluN2B plays a central role in LTP. This interaction was shown to support the specific activation of the ERK/MAPK pathway, which is critical in long-term memory. Meanwhile, LTP induction leads to the transient exit of PSD95 from the spine. Finally, NMDAR activation was shown to activate calpain, leading to the cleavage of several substrates including the cytoplasmic tails of GluN2 subunits.
The molecular dissection of these dynamic interactions in the context of spine remodeling is challenged by the limited resolution of optical microscopy. In this study, we combined Fluorescence Lifetime Imaging (FLIM) and Forster Resonance Energy Transfer (FRET) with biochemical techniques to investigate the implication of calpain in synaptic signaling and remodeling in cultured hippocampal and cortical neurons.
We first examined the interaction between PSD95 and the NMDAR subunits with FRET-FLIM using GFP and mCherry as donor/acceptor tags. Our results indicate that PSD95 interacts with the NMDAR in spines, but that synaptic activation of NMDARs, produced by a cLTP protocol, leads to the dissociation of this interaction. Blocking calpain activation prevented this dissociation, suggesting that NMDAR activity triggers a cleavage process in spines. Biochemical analyses from synaptic fractions of cortical neurons indicated that synaptic NMDAR activation leads to the cleavage GluN2B, but not to PSD95.
To examine the role of calpain-mediated cleavage of GluN2B on synaptic signaling and remodeling, we measured the impact on ERK phosphorylation and spine remodeling. Our results indicate that blocking calpain activity or removing putative calpain cleavage sites on GluN2B both prevented activity-dependent phosphorylation of ERK, spine volume increase. Our experiments thus suggest that synaptic activation of NMDARs leads to the cleavage of GluN2B by calpain which supports synaptic plasticity.

Disclosures: F. El gaamouch: None. **S. Labrecque:** None. **M. Lemieux:** None. **B. Tournier:** None. **K. Dore:** None. **P. De Koninck:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 233.11/G49

Topic: B.08. Synaptic Plasticity

Title: Membrane-to-cytosol translocation of activated AKT kinase disrupts expression of Long-Term Potentiation

Authors: *I. MICHAELEVSKI, Y. PEN, A. SHEININ, N. BOROVOK;
Tel Aviv Univ., Tel Aviv, Israel

Abstract: Background: Serine/threonine kinase AKT/PKB plays a fundamental role in a wide variety of cellular functions, including protein synthesis, regulation of cell survival, metabolism and proliferation. In neuronal cells, AKT has been shown to be involved in neuronal cell development, axonal growth and synaptic plasticity. Recently, a further downstream mechanism of AKT involvement in long-term depression (LTD) prevention and late phase long-term potentiation (LTP) has been revealed. Moreover, AKT is also known to be involved in early phase LTP; however no solid knowledge has been accumulated regarding the downstream effectors mediating AKT's effects on LTP. Here, we present our preliminary findings describing a mechanism of AKT's effect on LTP. Results: Using different regimes for application of the AKT inhibitor A6730 (30 min drug exposure before high frequency stimulation (HFS), drug for 30 min before and 20 min after HFS, drug for 20 min 30 min after HFS), to acute hippocampal slices trained with the HFS paradigm, we observed that AKT regulates LTP induction and expression without affecting its maintenance. Further, we delivered an AKT activator to combine with inhibitors of various signaling pathways to prevent AKT activation-induced effect on LTP and to characterize a potential downstream mechanism of LTP regulation. Unexpectedly, SC79 (activator of AKT), which also prevents AKT translocation to the plasma membrane, induced a significant decrease in basal synaptic activity and in expression of LTP. Conclusions: We consider that translocation of AKT towards the plasma membrane is necessary for regulation of synaptic activity and LTP. Moreover, AKT related LTP expression is not dependent on downstream cytosolic factors, but is mediated via direct effects of AKT on post-synaptic density components and glutamate receptors.

Disclosures: I. Michaelevski: None. **Y. Pen:** None. **A. Sheinin:** None. **N. Borovok:** None.

Poster

233. LTP: Kinases and Intracellular Signaling

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Program#/Poster#: 233.12/G50

Topic: B.08. Synaptic Plasticity

Support: NHRI-EX100-9618NI

NSC101-2321-B-006-024

Title: Oxytocin promotes long-term potentiation by enhancing epidermal growth factor receptor-mediated local translation of protein kinase Mζ

Authors: Y.-T. LIN[1], *Y.-C. LIU[2], C.-C. HUANG[1], K.-S. HSU[1];
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Abstract: In addition to triggering the birthing process and milk release, the hypothalamic neuropeptide oxytocin (OXT) plays an important role in the regulation of complex social cognition and behavior. Previous work has shown that OXT can regulate hippocampal synaptic plasticity and improve hippocampus-dependent cognitive functions in the female mice, but the underlying mechanisms remain largely unclear. Here, we demonstrate that OXT promotes the maintenance of long-term potentiation (LTP) induced by one train of tetanic stimulation (TS) in the CA1 region of hippocampal slices from both nulliparous female and male rats through a previously unknown mechanism involving OXT receptor (OXTR)-dependent and epidermal growth factor receptor (EGFR)-mediated local translation of an atypical protein kinase C isoform, protein kinase Mζ (PKMζ), in dendrites. Using pharmacological and biochemical approaches, we show that both the conventional OXTR-associated signaling pathway (Gq/11-coupled phospholipase C) and the transactivated EGFR downstream signaling pathways (phosphatidylinositol 3 kinase and extracellular signal-regulated kinase 1/2) are involved in the regulation of OXT. In addition, OXT stimulates local dendritic PKMζ mRNA translation via activation of a mammalian target of rapamycin-regulated mechanism. Furthermore, blockade of OXTR results in a modest decrease in the ability to maintain late-phase LTP induced by three trains of TS. These results reveal a novel OXTR-to-EGFR communication to regulate the new synthesis of PKMζ, which functions to promote the maintenance of LTP at hippocampal CA1 synapses.

Disclosures: Y. Lin: None. **Y. Liu:** None. **C. Huang:** None. **K. Hsu:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

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Topic: B.08. Synaptic Plasticity

Support: NIH MH063232

NIH NS078291

NIH NS007491

Title: Regulation of striatal CaMKII by extracellular calcium influx

Authors: *J. C. GANDY, R. COLBRAN;
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Abstract: Normal habit learning, motor control, and decision making requires striatal GABA-ergic medium spiny neurons (MSNs) to integrate excitatory inputs with modulatory inputs from other basal ganglia structures. Extracellular calcium influx into striatal MSNs contributes to these functions by regulating a multitude of processes including ion channel activity and synaptic plasticity. Coupling of calcium influx to synaptic regulation occurs through the calcium-dependent signaling proteins, such as Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Calcium promotes CaMKII autophosphorylation at Thr286, which results in sustained calcium-independent CaMKII activity after the calcium signal dissipates. Activated CaMKII can translocate to the postsynaptic density, phosphorylate synaptic proteins, and regulate NMDAR-dependent LTP in the hippocampus. CaMKII is also expressed in both direct (D1R) and indirect (D2R) pathway MSNs, modulates endocannabinoid dependent synaptic depression, and has a higher basal autophosphorylation at Thr286 as compared with other brain regions. However, little is known about the mechanisms regulating striatal CaMKII. Therefore, we acutely isolated striatal slices from adolescent mice, treated them pharmacologically, and then homogenized punches of dorsal striatum for immunoblotting using antibodies that detect Thr286 autophosphorylation as an index of CaMKII activation. We found that blocking spontaneous neuronal excitability with the voltage gated sodium channel blocker TTX failed to change basal CaMKII activity. However, chelation of extracellular calcium with BAPTA robustly decreased CaMKII autophosphorylation at Thr286 in a time dependent manner. This was coupled to significant decreases in the phosphorylation of Ser1303 in the NMDA receptor GluN2B subunit and Ser-831 in the AMPA receptor GluA1 subunit, which are targets of active CaMKII. Calcium depletion also promoted total CaMKII re-localization to non-synaptic subcellular fractions. In order to identify the calcium permeable ion channels that promote basal CaMKII activation, we

selectively modulated L-type voltage gated calcium channels (LTCCs) and T-type voltage gated calcium channels (TTCCs). Our preliminary findings show these channels differentially regulate basal CaMKII autophosphorylation at Thr286. Our ongoing studies are exploring the roles of these channels in CaMKII regulation in specific striatal MSN cell types through immunohistochemical studies. Together these studies will elucidate signaling mechanisms coupling calcium entry to regulation of striatal function.

Disclosures: J.C. Gandy: None. **R. Colbran:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

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Program#/Poster#: 233.14/G52

Topic: B.08. Synaptic Plasticity

Support: (FQRNT) Fond Québécois de la Recherche sur la Nature et les Technologies

(NSERC) Natural Sciences and Engineering Research Council of Canada

(CFI) Canada Foundation for Innovation

Title: A FRET-FLIM-STED approach to dissect protein interactions at nanoscale: Localizing CaMKII interactions within dendritic spines

Authors: *C. TARDIF, D. CÔTÉ, P. DE KONINCK;
Univ. Laval, Québec, QC, Canada

Abstract: The specific localization of protein interactions in the synaptic area, such as synaptic receptors and signaling proteins, can impact on the signaling cascade implicated in learning and memory. Knowing at nanoscale the position of those interactions inside and around the post-synaptic density could provide insights on the role of their partnership. This is particularly interesting in the context of synaptic plasticity, more specifically long term potentiation and depression (LTP & LTD). Optical methods using Fluorescence Lifetime Imaging (FLIM) to quantify Foster Resonant Energy Transfer (FRET) are useful to study protein interactions. FRET-FLIM approach provides limited spatial resolution due to the diffraction of light, particularly for studying interactions in synaptic domains, where the spine size is in the order of 0.5 μm. Super resolution methods, for instance STimulated Emission Deplession (STED), have been developed to beat this resolution limit. We combined STED with FRET-FLIM technique to study molecular interactions within dendritic spines at nanoscale resolution. We built a STED

microscope that uses a pulsed supercontinuum laser on which we added a Time Correlated Single Photon Counting (TCSPC) device to perform FRET-FLIM method. This microscope currently achieves an x/y resolution of 60 nm. To measure protein interactions, we used an immuno-FRET approach exploiting ATTO dyes, (e.g ATTO-594 as a donor and ATTO-647N as an acceptor). We first performed an immuno-FRET-FLIM-STED approach on a known, activity-regulated, interacting pair of proteins in cultured hippocampal neurons: The Calcium/Calmodulin-dependent protein kinase β (β CaMKII) and actin filaments (F-actin). We demonstrated the effectiveness of the method to resolve βCaMKII-F-actin interaction within subdomains of spines and dendrites. We are now investigating the interaction of αCaMKII with the N-methyl-D-aspartate receptor (NMDAR) under different levels of synaptic activity and cLTP conditions. Localizing the interaction of NMDAR with αCaMKII at nanoscale should help understanding the mechanisms underlying NMDAR-dependent synaptic plasticity.

Disclosures: C. Tardif: None. **D. Côté:** None. **P. De Koninck:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

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Program#/Poster#: 233.15/G53

Topic: B.08. Synaptic Plasticity

Support: PAPIIT IN212013

Title: Effects of ZIP infusion on the persistence of *in vivo* hippocampal mossy fibers synaptic plasticity

Authors: V. A. CÁCERES-CHÁVEZ, *M. L. ESCOBAR;
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Abstract: Persistence is a characteristic attribute of long-term memories. However, little is known about the molecular mechanisms that mediate this process. Recent experimental evidence shows that the persistence of some forms of long-term memory and long-term potentiation (LTP) can be disrupted with the administration of the atypical protein kinases C (aPKCs) inhibitor ZIP (ζ-pseudosubstrate inhibitor protein, also named Myr-aPKC-Pseudosubstrate peptide). In these sense it has been reported that ZIP administration reverses the late-phase of hippocampal LTP on the perforant and Schaffer collaterals pathways, but this effect has not been explored on the atypical hippocampal mossy fibers (MF)-CA3 pathway that exhibit an NMDA-receptor independent form of LTP. In a similar manner, the relationship between ZIP effects and morphological synaptic modifications that have been proposed to underlie memory persistence

has not been clearly observed in the adult brain. Our previous studies show that in vivo delivery of high-frequency stimulation (HFS) sufficient to induce LTP at the MF pathway elicits MF structural reorganization at the striatum oriens of CA3 area seven days after HFS. In the present study we administrated acute microinfusions of ZIP in the hippocampal CA3 area of adult rats during the late-phase of in vivo MF-CA3 LTP, with the purpose to evaluate the participation of aPKCs on the persistence of this atypical form of LTP as well as on its concomitant presynaptic structural reorganization. Our results show that ZIP inhibition of aPKCs activity neither reverses MF potentiation nor modifies its concomitant structural reorganization, even when it is able to reverse the perforant pathway LTP in our own preparations. These findings suggest that synaptic plasticity persistence on these atypical synapses does not requires of aPKCs activity in the same degree as other expressions of synaptic plasticity.

Disclosures: V.A. Cáceres-Chávez: None. **M.L. Escobar:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

Location: Halls B-H

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Program#/Poster#: 233.16/G54

Topic: B.08. Synaptic Plasticity

Support: CREST-JST

JSPS

MEXT

Title: Nonlinear decoding and asymmetric representation of neuronal input information by CaMKIIα and calcineurin

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Abstract: The nervous system adapts to a fluctuating environment through activity-dependent modulation of neuronal properties such as synaptic plasticity. The direction and extent of such sustainable modulation are determined by the stimulus parameters, suggesting that the biochemical machineries in synapses can readily compute the input information. Previous studies have suggested that Ca2+- and calmodulin-dependent kinase II (CaMKII) and calcineurin play

key roles in these decoding of input parameters and changes in spine morphology.
However, several important theoretical postulates underlying the role of CaMKII and calcineurin during synaptic plasticity_e.g. that CaMKII in spines functions as a high-frequency input detector or that calcineurin is uniquely activated by low-frequency stimulation_remain untested in living neurons. In particular, evidence is lacking whether distinct sets of incoming glutamate stimulation parameters can be transformed into a differential spatio-temporal activation patterns of the Ca2+-sensitive biochemical effectors. Furthermore, in spite of some models proposing a critical role of the calcineurin - inhibitor-1 - protein phosphatase-1 pathway in regulating CaMKII activity during plasticity, these ideas were not directly examined in hippocampal neurons.
To address these issues, we developed dFOMA (dual FRET with optical manipulation) imaging that permitted simultaneous measurement of two independent FRET probes along with local uncaging. Using this, we recorded CaMKIIα and calcineurin activities in hippocampal neurons, while varying glutamate uncaging frequencies. 5 Hz spine glutamate uncaging strongly stimulated calcineurin but not CaMKIIα, with little spine morphological change. In contrast, 20 Hz spine glutamate uncaging which induced spine growth activated both CaMKIIα and calcineurin, with distinct spatiotemporal kinetics. Treatment with a calcineurin inhibitor FK-506 did not significantly change the CaMKIIα responses triggered by either 20 Hz or 5 Hz glutamate uncaging, although it modestly increased baseline CaMKIIα activity. Higher temporal resolution recording in the soma revealed that CaMKIIα activity summed supralinearly and sensed both higher frequency and input number, thus acting as an input frequency/number decoder. In contrast, calcineurin activity summated sublinearly with increasing input number and showed little frequency-dependence, thus functioning as an input number counter.
These results provide evidence that CaMKIIα and calcineurin are fine-tuned to unique bandwidths and compute input variables in an asymmetric manner.

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Poster

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Core Research for Evolutional Science and Technology (CREST) from MEXT, Japan

Title: *In vitro* reconstitution of an erasable memory switch of CaMKII

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Abstract: Information is stored in our brain as changes in efficacy of synaptic connection, known as synaptic plasticity. In synaptic plasticity, a synaptic molecule 'Ca^{2+}/Calmodulin (Ca^{2+}/CaM)-dependent protein kinase II' (CaMKII) has been considered to provide a key mechanism. Autophosphorylation of CaMKII at T286 keeps CaMKII activated even after removal of Ca^{2+} stimulation, and CaMKII functions as a memory switch for maintenance of synaptic plasticity (Lisman 2002, Nat Rev Neurosci 3, pp. 175-190). Theoretical studies has predicted that both dephosphorylated 'OFF' and phosphorylated 'ON' states of CaMKII are stable in the presence of phosphatase, termed by 'bistability' (Zhabotinsky 2000, Biophys J 79, pp. 2211-2221; Okamoto 2000, Biol Cybern 82, pp. 35-47). Further, the interaction of CaMKII and *N*-methyl-*D*-aspartate (NMDA) receptor subunit GluN2B has been suggested to form a memory switch (Bayer 2001, Nature 411, pp. 801-805; Sanhueza 2013, Molecular brain 6:10). CaMKII has been shown to exhibit switch-like response to Ca^{2+} in vitro, but not to exhibit hysteresis, an essential property of memory (Bradshaw 2003, PNAS 100, pp. 10512-10517). Thus, it remains unclear whether CaMKII shows a memory switch with a hysteresis *in vitro*. Here, in an *in vitro* reconstitution system, we demonstrated that phosphorylation of CaMKII at T286 functions as a memory switch through interaction of a GluN2B-derived peptide. T286 phosphorylation clearly exhibited hysteresis, i.e., once Ca^{2+} stimulation reached a sufficient level to switch CaMKII to a phosphorylated 'ON' state, CaMKII remained the phosphorylated 'ON' state even if Ca^{2+} stimulation is lowered back below the threshold, indicating the memory function. This memory switch was achieved by the balance between CaMKII autophosphorylation and its dephosphorylation by protein phosphatase 1 (PP1), and the requirements of Ca^{2+} and PP1 concentration for the hysteresis showed reversibility and modifiability of the memory switch. Interestingly, T286 phosphorylation never propagated to neighboring molecules, and the CaMKII solution could have multiple steady states by mixing dephosphorylated 'OFF' and phosphorylated 'ON' states of CaMKII. Together with roles of a phosphosite of N2Bs, these findings demonstrate how the autophosphorylation of CaMKII at T286 contributes to our learning and memory.

Disclosures: H. Urakubo: None. **S. Ishii:** None. **S. Kuroda:** None.

Poster

233. LTP: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Title: PKA-dependent activation of presynaptically silent cortical synapses using a fluorescent probe

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Abstract: Neurotransmitter synaptic vesicle release has a compelling influence in neuronal network activity. It provides a resource and reserve capacity for neuronal network processing. The presence and awakening of silent synapses is one of the most dynamic resources in a neuronal network. We have previously observed a protein kinase A (PKA)-dependent activation of silent cortical synapses following administration of forskolin (FSK). It has been reported (Kaneko and Takahashi, 2004, J. Neurosci. 24:5202-5208) in the nerve terminal of the calyx of Held that cAMP facilitates release by activating the PKA-independent sensitive guanine nucleotide exchanging factor (GEF) also called (exchange protein directly activated by cAMP) Epac pathway. Therefore our objective here was to determine if a forskolin-induced increase in cAMP in our cultures also activated the Epac pathway. The experiments were performed on primary cultures of cortical neurons (E14-16) from C57BL/6J mice. To obtain direct measurements at presynaptic terminals two intracellular reporters were transfected at 7 DIV: Synaptophysin-pHluorin 2x (SypH2x) and G-Camp-3. G-Camp-3 reports on the internal calcium concentration and SypH2x on exocytosis and endocytosis. FSK (10µM) and Epac selective agonist 8-CPT2'-O-me-cAMP (100µM) were bath applied in the presence of AMPA receptor antagonist DNQX (10µM). Data analysis revealed that whereas FSK treatment did not change the internal calcium or endocytosis rate it increased the size of the readily releasable pool (RRP). The increase of the RRP confirms previous results of the effect of FSK on cortical synapses and suggests that activation of cAMP-dependent PKA is the necessary mechanism to uncover silent synapses.

Disclosures: T. Hikima: None. **G. Arbuthnott:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Z01ES102285

Title: Thyroid hormone signaling through the phosphatidylinositol 3-kinase acutely potentiates Schaffer collateral synapses on postnatal mouse hippocampal CA1 neurons through a presynaptic mechanism

Authors: F. MIZUNO, B. GLOSS, E. SCAPPINI, C. ERXLEBEN, *D. L. ARMSTRONG; Lab. Neurobio., NIEHS NIH, DURHAM, NC

Abstract: We reported previously that chronic blockade of thyroid hormone, 3,3′,5-triiodo-L-thyronine (T3), signaling through the phosphatidylinositol 3-kinase (PI3K) by a point mutation in the THRB gene, which produces TRbeta1 Y147F, interfered with the maturation and plasticity of Schaffer collateral synapses from CA3 pyramidal neurons onto CA1 neurons in the postnatal mouse hippocampus. Here we report that T3 also has rapid presynaptic effects on the strength of those synapses in slices from wild-type C57BL/6 mice. Thus, an 8 min application of 100 nM T3 rapidly increased the amplitude of evoked EPSCs by 50%, which is comparable to the increase produced by brief high-frequency stimulation, and this effect persisted for more than 1 hr. In contrast, the reverse isomer (3,3’,5’) of the hormone had no effect at the same concentration. T3 –induced potentiation did not depend on either postsynaptic calcium or activity, but it was blocked completely by 50nM wortmannin, a selective antagonist of PI3K. In slices from THRB mutant animals, the acute potentiating effect of T3 on synaptic responses in CA1 was eliminated completely, corroborating the involvement of PI3K signaling in this effect. Like other growth factors, such as BDNF, that potentiate hippocampal synapses through PI3K signaling, the effect of T3 appears to be primarily presynaptic. T3 increased the frequency of spontaneous EPSCs by 32% without affecting their amplitude. Moreover, T3 increased the paired pulse ratio by 15% at an inter-pulse interval of 50 ms. Further evidence for a presynaptic effect of thyroid hormone on synaptic plasticity was obtained by rescuing signaling in slices from the mutant. First we verified that the acute effect of thyroid hormone persisted for up to three weeks in cultured slices from wild type animals. Then we infected either the CA1 or the CA3 region of cultured slices from mutant animals with a neurotropic adeno-associated virus (AAV2/9) encoding the human synapsin promoter driving expression of wild type mouse TRbeta1 receptor linked with an IRES to green fluorescent protein (GFP). Ten days later, we recorded the effect of T3 on synaptic responses in CA1 neurons. Infection of CA3 neurons, which give rise to the Schaffer collaterals, totally restored T3-dependent potentiation of their synapses on CA1 neurons. In contrast, infection of the postsynaptic CA1 neurons restored T3-dependent potentiation of EPSC amplitude by only 10%. Thus, T3 signaling through PI3K stimulates postnatal synaptic plasticity in the mouse hippocampus by potentiating presynaptic glutamate release, and we hypothesize that TRbeta receptors in the mouse and human cortex may have similar effects.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: R01DA034979

Title: Molecular network models that can account for long-term plasticity and memory

Authors: *S. J. JALIL[1], T. C. SACKTOR[2], H. Z. SHOUVAL[1];
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Abstract: Memory and its cellular substrate, synaptic plasticity, can outlast by several orders of magnitude the dwell-time of the biomolecules in the synapses, which are assumed to be responsible for memories. Although the mechanism for such long lasting memories remains unknown, many types of memories are found to disappear following the injection of zeta inhibitory protein (ZIP) in small, targeted areas of the nervous system. Chronic pain and addictive behavior are affected in multiple studies when experimenters manipulated ZIP's target, PKMζ, an atypical protein kinase C isoform. The idea that ZIP erases memory by inactivating PKMζ has been contested, however, by the findings that animals with gene knockouts for this protein show normal memory, although more recent studies have shown compensation in such animals by the PKMζ paralogous isoform PKC/PKMι/λ (see Yao et al., Soc Neuro Abstr, 2013). Here, we examined, using a mathematical formulation, several possible types of minimal network models that can account for long-lasting memories and for different key experimental observations, such as the dependence of memories on de novo protein synthesis, memories' sensitivity to ZIP, and the effects of various pharmacological agents and mutants on memories. In addition, we examined the types of feedback mechanisms that are most likely to account for these experimental observations. Our analysis indicated that a model with auto-phosphorylation and differential synaptic dwell times (APDD) was the best match for multiple experimental results. Consequently, we were able to accurately model the experimental observations that a small amount of ZIP reduces late phase long-term potentiation (L-LTP), and a larger dose completely abolishes L-LTP.
We further examined several new experimental results, which question the importance of PKMζ,

as well as other observations which suggest that PKCλ (or PKMλ), the compensatory kinase, can also play a role in memory maintenance and can be up-regulated in PKMζ knock-out mice. We developed a model that includes these redundancies in the molecular substrates of memory and explore how this new parallel pathway model can account for the observed results. Finally, we propose an explanation for the apparent contradiction in the experimental data, and the enhanced robustness of the long-term memory resulting from the new parallel pathway model.

Disclosures: S.J. Jalil: None. **T.C. Sacktor:** None. **H.Z. Shouval:** None.

Poster

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Topic: B.08. Synaptic Plasticity

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NIH Grant R01 DA034970

Title: Conditional knockout of the PKC/PKMζ gene and acute translational blockade by PKMζ antisense show new synthesis of PKMζ protein is crucial for LTP and long-term memory

Authors: *P. TSOKAS[1], C. HSIEH[2], E. J. C. WALLACE[2], L. PAN[3], B. R. HARTLEY[3], A. TCHEREPANOV[2], A. I. HERNANDEZ[4], J. E. COTTRELL[5], A. A. FENTON[7], T. C. SACKTOR[6];
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Abstract: Protein kinase Mζ (PKMζ) is a brain-specific, constitutively active isoform of the atypical class of PKC that plays a key role in maintaining protein synthesis-dependent late-LTP (L-LTP) and long-term memory (LTM). During the induction of L-LTP or the consolidation of LTM in the hippocampus, PKMζ is synthesized from a PKMζ mRNA encoding a PKCζ catalytic domain without a regulatory domain. Using the selective atypical PKC inhibitor ZIP, the PKC

catalytic domain inhibitor chelerythrine, or a dominant negative mutant of PKMζ, previous studies have indicated that the persistent kinase activity of PKMζ is essential for the maintenance of L-LTP and LTM.
Because of the compensation in the constitutive PKMζ knockout (Yao et al., Abstr. Soc. Neuro., 2013), we used a conditional knockout (KO) approach to specifically delete the source of PKMζ mRNA. We injected Cre recombinase-expressing AAV into the hippocampi of adult mice in which exon 9, encoding the ATP-binding site of the PKC/PKMζ gene, is floxed. This selectively deletes PKMζ, because the hippocampus of wild-type mice expresses PKMζ, but not PKCζ. In conditional PKMζ-KO mice, a ~75% drop in the amount of PKMζ mRNA was found by qRT-PCR at 5 weeks post-injection. This was accompanied by a smaller (~12%) drop in PKMζ protein at 2-3 months post-injection, suggesting a long half-life for the kinase.
Immunocytochemistry of tetanized hippocampal slices from AAV-Cre-injected and contralateral control virus-injected hippocampi showed that conditional knockout prevented activity-depended synthesis of PKMζ protein in CA1 stratum radiatum. In the AAV-Cre-injected slices from conditional PKMζ-KO mice, tetanization induced only a decremental LTP (early-LTP), lasting ~2 hrs. Moreover, conditional PKMζ-KO mice were unable to consolidate spatial LTM of active place avoidance, 24 hr after training, but had intact short-term memory (STM). Control virus-injected mice had normal L-LTP and LTM.
These results were reproduced by acutely blocking de novo PKMζ protein synthesis, without affecting basal amounts of the kinase, using single intrahippocampal injections of PKMζ-antisense oligodeoxynucleotides (AS-ODNs). Acute AS-ODN injections blocked both new, activity-dependent PKMζ synthesis and L-LTP. AS-ODN injected 20 min prior to active place avoidance training blocked de novo PKMζ synthesis and prevented LTM consolidation measured 24 hr after training. STM was unaffected. SCR-ODN had no effect on new PKMζ synthesis, LTP, STM, or LTM.
These results demonstrate that, under physiological conditions or when compensation is avoided, newly synthesized PKMζ is crucial for the consolidation of late-LTP and spatial LTM.

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Poster

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Title: Compensation by PKCι/λ activation and PKMι/λ formation in PKMζ knock-out mice

Authors: *Y. YAO[1], P. TSOKAS[2], D. JOTHIANANDAN[1], A. TCHEREPANOV[1], P. VAN DE NES[1], T. SACKTOR[3];
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Abstract: PKCι/λ is a paralogue of PKCζ, the result of duplication of the single ancestral atypical PKC gene. The PKM form of PKCζ has been implicated in the maintenance of late-phase LTP and long-term memory. In rat hippocampal CA1 region, the amount of activated PKCι/λ also increases after induction of LTP (Kelly, J Neurosci, 27:3439, 2007), and it plays a critical role in early-LTP expression (Ren, et al., EMBO, 2013). Recent studies have found that constitutive PKC/PKMζ knock-out mice show unimpaired LTP and memory (Lee, et al., Nature. 493:416, 2013; Volk, et al., Nature, 493:420, 2013). Therefore, we investigated the possibility that PKCι/λ compensates for the loss of PKMζ. Biochemical studies show that in rat, mouse and human hippocampus, PKCι/λ is expressed in both full-length PKC form and a shorter PKM form consisting of the independent catalytic domain of the kinase. Moreover, the amount of both activated PKCι/λ and PKMι/λ in hippocampus are significantly increased in constitutive PKC/PKMζ knock-out mice, compared to their wild-type litter mates. Postsynaptic perfusion of activated PKCι/λ into rat hippocampal CA1 pyramidal cells caused a doubling of AMPA receptor-mediated EPSCs, similar to the potentiation observed by perfusing PKMζ. The PKMζ inhibitors, ZIP and chelerythrine, also inhibit PKCι/λ and PKMι/λ. Thus, PKCι/λ and PKMι/λ may compensate for the loss of function of PKMζ during the maintenance of LTP and long-term memory in constitutive PKC/PKMζ knock-out mice.

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Poster

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Title: Persistent increases of PKMζ correlate with the retention and duration of spatial long-term memory

Authors: *C. HSIEH[1], P. TSOKAS[1,2], B. R. HARTLEY[3], P. A. SERRANO[5], A. A. FENTON[6], T. C. SACKTOR[1,4];
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Abstract: PKMζ is a constitutively active and brain-specific atypical PKC isoform that plays an important role during long-term memory (LTM) storage. Overexpressing PKMζ enhances LTM (Shema et al., Science 331:1207 [2011]), and inhibiting PKMζ by overexpressing a dominant negative version of PKMζ or injecting ζ-pseudosubstrate inhibitory peptide (ZIP) disrupts LTM, even very long-term spatial memory 1 month posttraining (Pastalkova et al., Science 313:1141 [2006], Shema et al., Science 317:951 [2007], Shema et al., Science 331:1207 [2011]). Here we examined endogenous PKMζ expression at different time points after short- or long-term memory and correlated the amount of expressed PKMζ with LTM retention.
Adult male Long-Evans rats and C57BL/6J mice were trained by massed and spaced active place avoidance, respectively. Massed training consisted of eight 10-min-shock trials with 10 min inter-trial interval (ITI), and spaced training consisted of three 30-min-shock trials with 120 min ITI. Quantitative immunoblotting showed that dorsal hippocampal PKMζ increased by the end of the massed training phase, and 1 day after both massed and spaced training, compared to animals that had not received shock when placed in the apparatus. The increase in PKMζ remained at least 30 days after the spaced training. Moreover, the PKMζ level significantly correlated with 1 day LTM retention (Spearman's $\rho = -0.81$, $p < 0.01$), but not with the number of shocks during the training phase (Spearman's $\rho = 0.18$, $p = 0.63$). No PKMζ increase was detected after either short-term memory training or 1 day after the administration of the tethered shocks in which animals received the same number and timing of shocks as the avoidance-training group, but without association with spatial information. No long-term contextual fear conditioned behaviors, such as freezing, or locomotor activity change was observed in the tethered shock-training group. Immunohistochemistry demonstrated that the increased PKMζ after the spatial training localized in the strata pyramidale, radiatum, and lacunosum-moleculare in CA1 region at both 1 day and 1 month posttraining. These results support the hypothesis that persistently increased hippocampal PKMζ might be the molecular mechanism of the spatial LTM storage.

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Poster

233. LTP: Kinases and Intracellular Signaling

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Support: DA017392

MH081935

Japan Society for the Promotion of Science Postdoctoral Fellowships for ResearchAbroad

Title: Long-term potentiation at hilar mossy cell to dentate granule cell synapses

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Abstract: Hilar mossy cells (MCs) of the dentate gyrus receive inputs from dentate granule cells (DGCs) and project their glutamatergic axons back to DGCs, thereby establishing a positive feedback loop. While MCs have been implicated in epileptogenesis and could participate in some forms of memory, little is known about the dynamic properties of MC-DGC synapses. Here, we investigated whether MC-DGC synapses can undergo long-term, activity-dependent forms of plasticity. To this end, we performed whole-cell patch clamp recordings from DGCs in acute hippocampal slices of young-adult rats. Monosynaptic responses (excitatory postsynaptic currents, EPSCs) were monitored in the presence of the $GABA_A$ receptor antagonist picrotoxin (100 μM) while presynaptic MC fibers (MCFs) were activated by local extracellular stimulation (<150 μm from the recorded DGC) in the inner third of the dentate molecular layer (a.k.a. supragranular layer). We found that high frequency stimulation of MCFs in the presence of the NMDA receptor antagonist d-APV (50 μM) induced long-term potentiation (LTP) of MCF-EPSCs. This LTP was observed at both AMPAR- and NMDAR-mediated components of excitatory transmission, and was accompanied with a decrease in paired- pulse ratio and coefficient of variation, all of which suggest a presynaptic locus of expression. MCF-LTP was blocked by the PKA inhibitor H89 but not by the postsynaptic loading of the membrane impermeable PKA inhibitor peptide PKI_{6-22}. In addition, brief bath application of the adenylate-cyclase activator forskolin (50 μM, 10 min) induced a long-lasting enhancement of MCF-EPSCs that occluded synaptically-induced MCF-LTP. These results strongly suggest that presynaptic cAMP-PKA signaling is necessary and sufficient for MCF-LTP. Remarkably, postsynaptic loading of the Ca^{2+} chelator BAPTA (20 mM), or cyclopiazonic acid (CPA, 30 μM) to deplete intracellular Ca^{2+}stores, abolished MCF-LTP, indicating that a postsynaptic increase in Ca^{2+}

mainly arising from intracellular stores is required for induction. Thus, our findings provide evidence for an NMDA receptor-independent, cAMP/PKA-dependent form of LTP at MC-DGC synapses. The postsynaptic Ca^{2+} requirement for LTP induction together with a presynaptic mechanism of expression suggests the involvement of some form of retrograde signaling. While the precise role of MCs remains to be elucidated, the ability of the MC-DGC synapse to undergo a presynaptic, non-Hebbian form of LTP may have important implications for information processing in the dentate gyrus.

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Poster

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Title: Inositol polyphosphate multikinase enhances immediately early gene transcription via regulation of CBP

Authors: *R. XU[1], B. D. PAUL[2], R. TYAGI[2], F. RAO[2], A. B. KHAN[2], D. J. BLECH[2], P. GUHA[2], N. SEN[2], S. H. SNYDER[2];
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Abstract: Inositol polyphosphate multkinase (IPMK) is the rate-limiting enzyme in the formation of multiple higher inositol polyphosphate species, including both lipid-soluble PIP3 as well as water-soluble inositol tetrakisphosphates and inositol pentakisphosphate. Originally discovered in yeast as a transcriptional regulator of arginine metabolism, IPMK was subsequently shown to regulate chromatin remodeling complexes (Swi/Snf, ISWI, INO80, etc) in yeast via its metabolites. To date, however, the role of mammalian IPMK is less well understood. Conventional deletion of IPMK in mice is embryonic lethal at E9.5 with abnormal folding of the neural tube, precluding its study in neurons directly. Here, we generated brain-specific IPMK knockout mice using a nestin-Cre loxP system. Immediately early gene (c-fos, egr2, egr3, VGF, c-jun) induction in these mice are severely impaired after electroconvulsive shock, both at the mRNA and protein levels. Overexpressed and endogenous IPMK binds to, and recruits CBP to the c-fos promoter in both primary neurons and in mouse brain. Interestingly, rescue of IPMK knockout neurons with wild-type versus kinase-dead IPMK revealed that IPMK is able to enhance CBP recruitment to the c-fos promoter independent of its catalytic ability. Indeed,

overexpression of a dominant negative fragment of IPMK which blocks IPMK-CBP association decreased transcription of c-fos mRNA. Taken together, these results show that IPMK enhances immediate early gene transcription via recruitment of CBP.

Disclosures: R. Xu: None. **B.D. Paul:** None. **R. Tyagi:** None. **A.B. Khan:** None. **D.J. Blech:** None. **F. Rao:** None. **P. Guha:** None. **S.H. Snyder:** None. **N. Sen:** None.

Poster

233. **LTP: Kinases and Intracellular Signaling**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 233.26/H4

Topic: B.08. Synaptic Plasticity

Support: Wellcome-DBT India Alliance Intermediate Fellowship

NSF sponsored Center for Theoretical Biological Physics, Grant PHY0216576 and PHY0225630

Title: Effect of impaired neurotransmitter release on Long Term Potentiation

Authors: *S. NADKARNI[1], A. MADUSKAR[2], H. LEVINE[3];
[1]Computat. Neurobio. Lab., Indian Inst. of Sci. Educ. and Res. Pune, Pune, India; [2]Biol., Indian Inst. of Sci. Educ. and Research, Pune, Pune, India; [3]Dept. of Bioengineering, Rice Univ., Houston, TX

Abstract: Changes in Short-Term Plasticity (STP) leading up to modified Long-Term Plasticity (LTP) and synaptic dysfunction has been reported as a basis of initial pathological changes seen in Alzheimer's Disease (AD). Specifically, Rynanodine Receptors (RyRs) in the presynaptic terminal at the CA3-CA1 synapse have been implicated. However the exact chronology of signaling pathways that lead to aberrational LTP are difficult to identify experimentally. We have developed a biophysically detailed computational model of the CA3 presynaptic terminal in the hippocampus that includes the action of Ryanodine Receptors (RyRs) and Inositol Triphisphate Receptors (IP3). We simulate, within a range of physiologically relevant input frequencies, the effect of impending calcium release from the Endoplasmic Reticulum (ER) via the IP3 and RyR receptors on STP. Further we investigate how pathological excess calcium release from the ER affects neurotransmitter release profile. Our model suggests that the competing dynamics of neurotransmitter recycling compared to modified neurotransmitter release as a result of excess calcium release from the ER may lead to the depletion of the small readily releasable pool (~ 7 vesicles) in the CA3 terminal. The reduced Paired Pulse Facilitation

seen in experimental models of AD may thus be a consequence of altered calcium signaling from the RyRs. Further, we show how altered presynaptic calcium dynamics as seen in experimental models of AD lowers LTP.

Disclosures: S. Nadkarni: None. **A. Maduskar:** None. **H. Levine:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.01/H5

Topic: B.09. Network Interactions

Title: Humor associated mirthful laughter enhances brain EEG power spectral density gamma wave band activity (31-40Hz)

Authors: *L. S. BERK, P. PAWAR, C. ALPHONSO, N. REKAPALLI, R. ARORA, P. CAVALCANTI;
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Abstract: Humor associated mirthful laughter experiences (HAML) relate to various fundamental aspects of human behavior. Some of these experiences are characterized by modulating emotions, enhancing "higher mental activity", memory, sensory perception, consciousness and happiness. To date, no known electroencephalographic (EEG) study has been conducted to identify and differentiate Power Spectral Density (PSD) correlates across frequency bins1-40Hz, with particular interest of the 31-40Hz gamma band, for the comparative experience between the eustress HAML with a classic distress experience. It is known that gamma brain activity (GBA) is the fastest brainwave frequency with the smallest amplitude, however, GBA is associated with peak mental concentration ("in the zone"), memory enhancement and high levels of cognitive function. Purpose: This study measured EEG spectral power in 20 healthy university students, mean age 28±6.2, during HAML and stress experiences to differentiate PSD correlates across frequency bins1-40Hz. Methods: EEG activity was recorded from 9 scalp locations/channels F3, Fz, F4, C3, Cz, C4, P3, POz, and P4 using the EEG B-Alert 10X System™. In a cross-over design each subject was randomly administered (with an appropriate washout period) two separate 10 minute videos: Humor (America's Funniest Home Videos) and Stress (Movie - Saving Private Ryan). Results: Comparing HAML vs. stress, all nine channels showed greater Beta Band Activity (13-30Hz) (BBA) (p=0.01) and GBA (31-40HZ) (p=0.001) for HAML. Most significant was that Humor GBA was greater than Humor BBA (p=0.01) for all nine channels. Comparing HAML vs. Stress, Theta (3-7Hz) and Alpha (8-12Hz) for Parietal Left

(P3) vs. Parietal Right (P4) showed Humor greater in the P3 region than P4, for both bandwidths. Conclusions: These results indicate that HAML have correlates of marked changes in EEG PSD for specific bandwidths but most significantly for (GBA) across the whole hemispheric brain as measured by the B-Alert 10X System™. Other research suggests 1) GBA offers an adequate tool for studying cortical activation patterns during emotional processing and 2) GBA is suitable for EEG-based emotion classification of happiness and sadness. Our EEG findings support these findings and further suggest that HAML can increase PSD of GBA to potentially modulate states of "higher mental activity", sensory perception, enhancement of memory/recall and happiness. We suggest that PSD of GBA may be a functional neurofeedback marker of these states. Further research is being conducted to expand and elaborate these findings.

Disclosures: L.S. Berk: None. **P. Pawar:** None. **C. Alphonso:** None. **N. Rekapalli:** None. **R. Arora:** None. **P. Cavalcanti:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.02/H6

Topic: B.09. Network Interactions

Support: NIMH grant R01MH084038

NIMH grant R01MH099128

Title: Method for event-based cross-frequency coupling analysis of neural oscillations

Authors: *D. DVORAK[1], B. RADWAN[2], A. A. FENTON[2,1];
[1]Physiol. & Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Phase amplitude coupling (PAC) of neural oscillations was proposed as a possible mechanism for cross-scale organization of neural network activities. As an example, the phase of a slow rhythm like hippocampal theta oscillations modulates the amplitude of a faster rhythm like hippocampal gamma oscillations. To quantify this cross-frequency interaction, one has to use relatively long time windows (> 10 seconds). However, such long time windows are well beyond the relevant behavioral and physiological timescales for most cognitive events, and therefore provide only a global estimate of PAC, that is difficult to directly associate with cognitive events. In order to study PAC at sub-second time resolution, we developed a parameter-free data-driven algorithm, which considers fast frequency oscillations as time-

specific events rather than averaged power estimates obtained from long time windows. To test the performance of our algorithm we first compared it with a standard PAC algorithm (Tort 2010) for detecting individual oscillatory bands of phase-modulated oscillations. Second, we extracted features of the detected oscillations such as bandwidth, duration, power or number of oscillatory cycles and demonstrated frequency- and phase-specific modulation of these features and therefore their ability to capture ongoing cross-frequency interactions at the scale of single oscillations. Third, we compared the relationship between the modulation index obtained from the standard PAC algorithm with the single phase-modulated oscillations detected by our algorithm and show that increased modulation indexes correspond to higher numbers of phase-modulated oscillatory events, their increased power as well as their reduced phase variance.

Disclosures: D. Dvorak: None. **B. Radwan:** None. **A.A. Fenton:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.03/H7

Topic: B.09. Network Interactions

Support: NIMH grant R01MH084038

NIMH grant R01MH099128

Title: Event-based cross-frequency coupling of neural oscillations in the rodent hippocampus during cognitive events

Authors: *A. A. FENTON[1,2], D. DVORAK[3], B. RADWAN[1];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Physiol. & Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [3]SUNY Downstate Med. Ctr. / NYU-Poly, Brooklyn, NY

Abstract: It has been hypothesized that phase amplitude coupling (PAC) of neural oscillations might play a role in multiple brain functions such as cross-scale organization, selection of attention, routing the flow of information, memory processing, information coding and integration. To date, attempts to quantify PAC relied mainly on global estimates of cross-frequency relationships obtained from long time windows (> 10 seconds). In this framework, PAC is characterized as a statistical bias of the amplitude of the fast rhythm (such as hippocampal gamma oscillations) by phase of the slow rhythm (such as hippocampal theta oscillations). These estimates, however, provide only global information about the cross-frequency interaction but do not allow insight into the instantaneous changes in oscillatory

dynamics i.e. appearance of fast oscillations during single cycles of slow oscillation, that relate to neural function. In this study we present dynamic properties of individual phase-modulated oscillations obtained from rodent hippocampus (mouse and rat) during a variety of discrete behaviors, including i) pausing with and without vicarious trial-and-error, ii) switching between spatial reference frames, and iii) successful and failed place avoidance. The frequency-specific (i.e. slow gamma, fast-gamma and high frequency oscillation) characteristics of the individual phase-modulated oscillations associated with these cognitive-behavioral events are characterized for each set of events. Furthermore, the use of multiple, linearly spaced electrodes allowed linking of these ongoing oscillatory activity to specific layers around hippocampal CA1-CA3/DG axis.

Disclosures: D. Dvorak: None. **B. Radwan:** None. **A.A. Fenton:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.04/H8

Topic: B.09. Network Interactions

Support: NIH grant NS 065877

NIH grant NS 33310

Title: Gamma events and functional connectivity between brain areas

Authors: *A. BRAGIN, F. KHEIRI, J. ENGEL, Jr;
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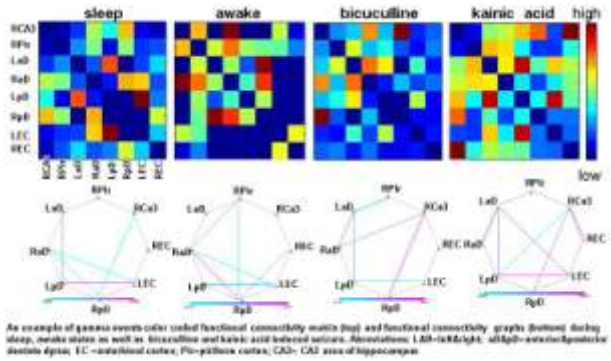
Abstract: In this study we investigated functional connectivity between brain areas by analysis of gamma activity. We have considered gamma activity as a sequence of events generated by local modules. We found that the size of these modules is within the range of 2-3mm. The temporal relationship between gamma events generated in different brain areas was estimated by calculation of perievent histograms. The strength of the peak in the histogram was measured using Shannon entropy followed by calculation of the connectivity index, which reflects the strength of functional connectivity between recorded brain areas. The spatial pattern of functional connectivity is represented by color coded matrices and connectivity graphs. Our data show that the spatial pattern of functional connectivity, to a certain degree, reflects morphologically defined connections between brain areas, but is unique for each individual animal. This spatial pattern is stable in a given behavioral state, but changes when animals move

from one behavioral state to another. Pharmacological manipulation of different types of synaptic transmission changes the spatial pattern of functional connections. This approach opens a new horizon for investigation of brain functional connectomics and may be applied for merging fMRI and electrographic data in investigation of functional properties of neuronal networks.



Disclosures: A. Bragin: None. **F. Kheiri:** None. **J. Engel:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.05/H9-DP3

Topic: B.09. Network Interactions

Support: Wellcome Trust Grant 087756/Z/08/Z

Title: Different mechanisms underlying feedforward and feedback influences on low and high frequency oscillations in visual attention

Authors: *M. BAUER, K. J. FRISTON, R. J. DOLAN;
UCL, London, United Kingdom

Abstract: Human and animal studies show that feedforward sensory signals and feedback influences from higher cortical areas modulate low frequency alpha-/beta- and high frequency gamma-oscillations in sensory cortex in a specific fashion: Alpha-/beta-frequencies are suppressed while gamma-frequencies are enhanced by stimulation and the attentional bias in the sensory cortex contralateral to the relevant stimulus location. We used two visual spatial attention paradigms, in one the task relevant stimulus feature was shown immediately with stimulus onset, in the other with an unpredictable delay and recorded MEG in humans. We show that 1) the timing of attentional alpha-/beta modulation generally differs from that of gamma

modulations in that the former commences before the relevant display onset whereas the latter always follows stimulation onset, 2) the frequency of the attentional modulation differs from both the resting state frequency of alpha oscillations as well as its suppression driven by feedforward stimulation in that the attentional modulation was concentrated between 10 and 20 Hz whereas the feedforward suppression revealed two clearly distinct peaks at around 10 and 20 Hz. Importantly, 3) the timing of the spatial attentional alpha-/beta-modulation strongly depended on the expected onset of the task-relevant stimulus feature and directly preceded this, independent from the onset of stimulation, whereas modulation of gamma more or less invariantly followed the onset of stimulation, independent from the onset of the task-relevant feature (i.e. either before or after presentation of the task relevant stimulus feature). These results reveal that different mechanisms contribute to feedforward vs feedback control of alpha-/beta-oscillations and in the attentional control or feedback regulation of alpha-/beta vs gamma-oscillations.

Disclosures: M. Bauer: None. **K.J. Friston:** None. **R.J. Dolan:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.06/H10

Topic: B.09. Network Interactions

Support: CIHR Team Research and Training Program in Sleep and Biological Rhythms

Title: Activation of thalamic δ-subunit containing $GABA_A$ receptors by hypnotics supports cortical deactivation *In vivo*

Authors: *L. MESBAH-OSKUI[1], B. A. ORSER[3,2,1], R. L. HORNER[2,1];
[1]Med., [2]Physiol., Univ. of Toronto, Toronto, ON, Canada; [3]Anesthesia, Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada

Abstract: Enhanced γ-aminobutyric acid (GABA)-ergic neural activity promotes natural sleep. Many hypnotics increase neural inhibition via interactions with binding sites on extrasynaptic δ-subunit-containing $GABA_A$ receptors (δ$GABA_A$R). The thalamus is a key structure in mediating cortical activity and controlling brain arousal. Activation of δ$GABA_A$R in the ventrobasalis complex (VB) of the thalamus elicits a tonic hyperpolarization *in vitro* that promotes a change in neural population activity consistent with brain deactivation. We examined the influence of δ$GABA_A$R activity in response to hypnotics in the VB on cortical activity. Experiments were conducted in 42 freely behaving δ$GABA_A$R knockout (*Gabrd*$^{-/-}$) and wild type (WT) mice.

Electrocortical activity was recorded during bilateral microperfusion of artificial cerebrospinal fluid (aCSF), 10μM and 50μM THIP (a δGABA$_A$R-preferring agonist), and 10μM and 30μM etomidate into the VB. Mice of each genotype also served as time-controls, receiving only aCSF. In WT mice, 50μM THIP at the VB increased 1-4Hz electrocortical activity in non-rapid eye movement (NREM) sleep and waking. Delivery of 50μM THIP to the VB also influenced state dynamics, with transitions into NREM and REM sleep occurring more rapidly. Sigma power (10-15Hz) and spindle density (7-14Hz) during NREM sleep were also reduced. Importantly, no such changes occurred with THIP in *Gabrd*$^{-/-}$ or time-control mice. Surprisingly, 10μM etomidate in the VB of WT and *Gabrd*$^{-/-}$ mice decreased 1-4Hz electrococritcal activity and increased sigma oscillatory power. Consistent with the increased sigma power, spindle incidence was markedly increased, as was average spindle duration. Transitions into NREM and REM sleep were unaffected; however, there was an increase in the incidence REM sleep in both strains of mice. Interestingly, 30μM etomidate at the VB promoted slow, 1-4Hz, oscillations in WT and *Gabrd*$^{-/-}$ mice. Sigma power, spindle incidence and duration were dampened and transitions into NREM and REM sleep occurred more rapidly. Importantly, all of the differences seen with 30μM etomidate at the VB were less pronounced in *Gabrd*$^{-/-}$ relative to WT mice. Since prominent delta (1-4Hz) oscillations are a hallmark of sleep and anesthetic states, these data suggest that δGABA$_A$R at the VB promote cortical deactivation, and as such are positioned to contribute to the induction of sleep and sedation. Our findings also indicate that intermediate levels of etomidate (consistent with amnestic brain concentrations) in the thalamus elicit changes in cortical activity that are not primarily mediated through δGABA$_A$R.

Disclosures: L. Mesbah-Oskui: None. **B.A. Orser:** None. **R.L. Horner:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

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Program#/Poster#: 234.07/H11

Topic: B.09. Network Interactions

Support: NIH Grant R01MH096482-01

NIH Grant 5R21CA159470-02

Title: Electrophysiologic correlates of resting-state fMRI networks

Authors: *C. D. HACKER[1], A. SNYDER[2], M. PAHWA[2], M. SHARMA[2], D. BUNDY[2], A. DAITCH[2], N. SZRAMA[2], K. BANDT[2], T. MITCHELL[2], M. CORBETTA[2], E. LEUTHARDT[2];

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Abstract: Investigations of ongoing brain activity in the resting state (in the absence of salient sensory and motor events) have revealed spontaneous correlations within widely distributed brain regions known as resting-networks (RSNs). Each RSN is putatively associated with a specific function such as vision, motor control and language. However, the physiological underpinnings of RSNs are incompletely understood. RSNs have thus far been studied primarily with non-invasive neuroimaging techniques (specifically functional MRI). We combined functional MRI and invasive electrophysiology to investigate the electrophysiological correlates of resting state networks.
We investigated the correspondence between electrocorticographic (ECoG, brain surface potential) recordings and RSNs defined using fMRI in patients undergoing invasive monitoring prior to surgical treatment of epilepsy (N=15). Electrodes were segmented from a CT image. Physical brain distortion due to grid implantation was corrected by projection to a pial surface segmentation generated with FreeSurfer. EPI distortion in BOLD data was corrected with retrospective application of a mean field map. Voxel-wise estimates of RSN identity were computed with a multi-layer perceptron (MLP). The MLP had previously been trained to associate fMRI correlation maps of canonical seed regions (generated from a meta-analysis of task responses) with 7 RSNs. We sampled RSN values onto electrodes, weighting each voxel as a dipole and thus accounting for distance and cortical surface angle relative to the electrode.
We determined ECoG-fMRI RSN correspondence by contrasting correlations of ECoG signals between groups of electrode pairs within vs. across fMRI-defined RSNs. We found a significant correspondence at the group level for the dorsal attention, motor, visual, language, and default mode networks. This effect was present from infra-slow to high alpha-range frequencies (0.5 to12 Hz) for raw timeseries. These results extend previous work demonstrating ECoG correlates of the sensorimotor RSN below 4 Hz (He, 2008). We applied the same analysis to infra-slow (< 0.5 Hz) fluctuations of band-limited power (BLP) at carrier frequencies ranging from theta (3-5 Hz) to high gamma (up to 150 Hz). Greater within vs across RSN correlations were sharply tuned in carrier frequency for some RSNs and much more broadly tuned for others. In general, the patterns of BLP correspondence as a function of carrier frequency for different RSNs were highly variable across subjects compared to those of raw ECoG timeseries.
He et al. PNAS 2008; 105:16039-44

Disclosures: C.D. Hacker: None. **A. Snyder:** None. **M. Pahwa:** None. **M. Sharma:** None. **D. Bundy:** None. **A. Daitch:** None. **N. Szrama:** None. **K. Bandt:** None. **T. Mitchell:** None. **M. Corbetta:** None. **E. Leuthardt:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.08/H12

Topic: B.09. Network Interactions

Support: NIH 1P50MH094271

Title: Enhanced cortical gamma oscillations and cognitive impairment by selective disruption of inhibition within parvalbumin-networks

Authors: *T. K. HENSCH[1,2], H. H. LEE[2];
[1]Harvard Univ., CAMBRIDGE, MA; [2]Boston Children's Hosp., Boston, MA

Abstract: Gamma oscillations emerge from an intricate interplay between fast-spiking inhibitory interneurons and their pyramidal cell targets. Vulnerability of these parvalbumin (PV)-positive cells in turn underlies various hypotheses of schizophrenia (glutamatergic, dopaminergic, neurodevelopmental, NRG/ErbB4, oxidative stress). Causal relationships between gamma oscillations and cognitive deficits remain elusive. While sensory-evoked oscillations are often reduced in schizophrenia, spontaneous gamma power (30-80 Hz) is paradoxically increased. Gamma band abnormalities may represent a neurobiological endophenotype or simply be a very sensitive readout or epiphenomenon of any perturbed cortical network function. Here, we specifically examined a role for PV-network connectivity in gamma oscillations. We disrupted the major $GABA_A$ receptor subunit, alpha1, within PV-cells in adolescence (alpha1$^{f/f}$ x PV-Cre^{+} mice) and observed elevated levels of spontaneous EEG gamma power across different regions of neocortex (prefrontal: 343±34%, N=6; somatosensory: 190±16%, N=7; visual: 679±87%, N=3, as compared to WT littermates). The prefrontal EEG abnormality was notably associated with behavioral deficits including social memory, novel object recognition, attention-based exploratory activities and an elevated level of pre-pulse inhibition. NMDA receptors on fast-spiking cells may contribute to gamma band synchrony. We confirmed that EEG gamma oscillations are induced in mice by ketamine (171±19% above baseline, N=5), a non-competitive antagonist often linked to cognition and information processing deficits in drug abusers. This ketamine effect on gamma oscillations was largely occluded in alpha1$^{f/f}$ x PV-Cre^{+} mice (118±10%, N=4). Instead, the non-selective allosteric $GABA_A$ receptor modulator, diazepam, suppressed high-range gamma oscillations (60-80Hz) (51±2%, N=6) but not in WT mice (99±3%, N=3), while slightly elevating low-range gamma (30-60Hz) both in mutant (126±14%, N=6) and WT mice (154±15%, N=3). This diazepam treatment further rescued social memory deficits in the alpha1$^{f/f}$ x PV-Cre^{+} mice. Taken together, GABAergic signaling within the PV-network (through alpha1 subunits) normally dampens gamma oscillations and mimics some aspects of cognitive impairment when disrupted. Gamma oscillations may provide a temporal structure for activity-dependent synaptic refinement to take place. Periadolescent disturbance of

PV-circuit connectivity, which normally drives critical periods of brain plasticity, may trigger aberrant synaptic pruning and associated cognitive deficits.

Disclosures: T.K. Hensch: None. **H.H. Lee:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.09/H13

Topic: B.09. Network Interactions

Support: NSERC 249861

Title: Frontal field stimulation promotes cortico-hippocampal interactions during slow-wave activity

Authors: *A. GREENBERG[1], T. WOLANSKY[2], C. T. DICKSON[3];
[2]Dept. of Psychology, [3]Dept. of Psychology, Ctr. for Neuroscience, Dept. of Physiol., [1]Univ. of Alberta, Edmonton, AB, Canada

Abstract: The signature event of slow-wave sleep (SWS) is the neocortical large amplitude slow oscillation (SO; ~1Hz) that is composed of alternating periods of highly active (UP) and silent (DOWN) states at the neuronal level. On each wave, the SO is generated at a local focus in the neocortex, and is propagated across the cortical network, and even to the hippocampus, in various directional patterns. This potential interaction between the neocortex and hippocampus make the SO a favourable candidate for mediating sleep-dependent declarative memory consolidation. In fact, it has been previously shown that the application of transcranial electrical stimulation at the SO frequency to human subjects during SWS improved declarative memory recall the following day. However, influence of electrical stimulation on cortico-hippocampal spatiotemporal interactions during SO remain unknown. Here, we show in urethane anesthetized rats that rhythmic electrical field stimulation applied to the anterior pole of the cortex entrains cortical and hippocampal local field and cellular activity and biases the spontaneous propagation of the SO in an anterior-to-posterior direction across the cortex and that subsequently appears in the hippocampus. Field stimulation also enhanced long-range gamma synchrony across both cortico-cortical and cortico-hippocampal regions, an effect that transiently persisted following cessation of stimulation. Furthermore, field stimulation also increased the incidence of hippocampal ripples which were modulated by the phase of stimulation and appeared in a non-overlapping fashion with gamma activity. These results shed new insights into the potential

neurophysiological mechanisms supporting plastic processes such as memory consolidation during SWS.

Disclosures: A. Greenberg: None. **T. Wolansky:** None. **C.T. Dickson:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.10/H14

Topic: B.09. Network Interactions

Title: Dynamical features of interictal spikes in the human brain undergoing anesthesia

Authors: *G. A. CECCHI[1], L. ALONSO[2], M. MAGNASCO[2], A. PROEKT[2];
[1]Biometaphorical Computing, IBM Res., YORKTWN HGHTS, NY; [2]The Rockefeller Univ., New York, NY

Abstract: We collected data from ECoG measurements (invasive electrocorticography) in epileptic human patients. These measurements are used by clinicians as part of a procedure prior the approval of surgery. Each electrode is placed on a grid over the exposed cortex and registers the synchronized activity of thousands of postsynaptic potentials. Since the procedure is invasive, the spatial resolution of this measurements is considerably higher than noninvasive EEG.

It is widely believed that there are pathological waveforms in these recordings which can be associated with epileptic seizures. Moreover, interictal spikes are used as a diagnostic tool for determining the spatial location of the seizure focus. The dynamical connection between interictal spikes and the onset of seizures remains elusive [1]. In this work we apply a nonlinear lter to detect interictal spikes in ECoG recordings. We nd highly stereotyped waveforms with at least two timescales which occur when the subject is undergoing anesthesia. The occurrence of these events is highly correlated amongst sites on the grid.

As the subjects undergo anesthesia, several dynamical features of the recorded timeseries are a ected. Previous work by our group and others suggest that the brain operates in a dynamically critical regime [2, 3]. Here, we perform a moving VAR analysis on our dataset and show that the spectral properties of the linear stability matrix change signicantly. When the subject is awake, many modes are poised close to the instability. Finally, when the subject is unconscious, the system becomes more stable.

This empirical evidence is consistent with the idea that dynamical systems capable of optimally performing computations should be poised at the critical regime [4].

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[3] E. Gireesh and D. Plenz, PNAS., 105 (2008)
[4] C. Langton. Phys. Rev. E., 42 (1990)

Disclosures: G.A. Cecchi: None. **L. Alonso:** None. **M. Magnasco:** None. **A. Proekt:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.11/H15

Topic: B.09. Network Interactions

Support: German Research Society (DFG, SFB-TR3, TP C3, to E.J.S.

Title: Slow (DC) Potentials of the living human brain

Authors: *E.-J. SPECKMANN[1], A. GORJI[2];
[1]Univ. Munster, Munster 48149, Germany; [2]Epilepsy Res. Ctr., University Munster, Germany

Abstract: DC potentials of the brain comprise sustained displacements of the baseline as well as fluctuations between 0 Hz and 100 Hz including the frequency range of the conventional EEG. Neurons, glial cells and blood-brain barriers forming functional units may act as a compound generator of DC potentials. Several investigations both on animal and man have already shown that recordings of DC potentials of the brain provide an objective access to the understanding of higher brain functions as well as pathophysiology of different neurological disorders. The slow components of neocortical DC potential shifts reflect changes in the level of excitation and excitability of the cortex. Epileptiform field potentials, either focal or generalized discharges, are always associated with characteristic deviations of the DC potential in different animal model of epilepsy. Negative DC fluctuation was also observed accompanied by ictal discharges in human brain. In animal experiments, a reduction of the oxygen content of the brain evokes negative DC displacements in both in vivo and in vitro models. The same negative DC deflections were reported in patients suffering from hypoxic brain injuries. Spreading depression, a negative DC deflection, has been shown to play crucial role in aura phase of migraine and cerebrovascular diseases. Psychophysiological studies revealed that DC potentials are highly sensitive to different information processing and to personal characteristics. It seems promising to extend DC potentials studies to provide further insights into mechanisms of brain function and dysfunction.

Disclosures: E. Speckmann: None. **A. Gorji:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.12/H16

Topic: B.09. Network Interactions

Support: Wellcome Trust 092994

Title: The effect of ventral tegmental area (VTA) stimulation on slow wave sleep Up-Down states in the prefrontal cortex of anaesthetized rats

Authors: *S. GRETENKORD[1], S. E. GARTSIDE[1], A. REES[1], M. A. WHITTINGTON[2], F. E. N. LEBEAU[1];
[1]Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [2]Hull York Med. Sch., Univ. of York, York, United Kingdom

Abstract: Cognitive impairment is a key feature of schizophrenia that is associated with abnormal beta (15-30 Hz) and gamma (30-90 Hz) frequency oscillations during wakefulness (Uhlhaas et al., 2008). Sleep abnormalities, however, are also common in schizophrenia where changes in slow wave sleep (SWS) have been linked to cognitive deficits (Manoach et al., 2010, Ramakrishnan et al., 2012). SWS is a brain state that manifests in the local field potential (LFP) as a slow oscillation (<1 Hz) of alternating "Up" states (periods with high frequency gamma activity) and "Down" states (periods with lower frequency activity). The VTA dopamine system is implicated in schizophrenia as well as in sleep regulation.

We have assessed the effect of VTA stimulation on Up-Down states in the prelimbic cortex in male hooded-Lister rats anaesthetized with urethane (1.5-1.8 g/kg i.p.). VTA stimulation changed the LFP from the low frequency-high amplitude SWS pattern to a high frequency-low amplitude rhythm within a few seconds of the onset of the stimulation. This effect was reproducible and lasted for up to 30 seconds beyond the end of the stimulation. Systemic administration of the dopamine $D_{1,5}$ receptor antagonist SCH23390 (0.6 mg/kg i.p.) either delayed the transition or completely blocked the LFP response to the VTA stimulation. This blockade could be reversed by subsequent systemic administration of the $D_{2,3}$ antagonist sulpiride (10 mg/kg i.p.).
There was also a trend for the VTA stimulation to increase Up-state duration (comparing a post-stimulation to a pre-stimulation period): an effect which also appeared to be D_1 receptor mediated.

Our LFP recordings indicate a strong modulation of the prefrontal slow wave activity by dopamine which is $D_{1,5}$ mediated. The effect of sulpiride suggests that electrically-evoked dopamine release in the prefrontal cortex is sensitive to D_2 autoreceptor modulation such that blockade of D_2 (auto)receptors can overcome the effects of postsynaptic $D_{1,5}$ blockade. Our results are consistent with the results of Lewis and O'Donnell (2000) who reported that dopamine release induced by VTA stimulation induces a transition to a long-lasting Up-state in prefrontal pyramidal cells which was $D_{1,5}$ receptor mediated.
A disruption of dopaminergic activity, as apparent in schizophrenia, might also disrupt slow wave sleep in humans. We hypothesize that such a disruption could explain some of the cognitive deficits seen in schizophrenia patients.

Disclosures: S. Gretenkord: None. **S.E. Gartside:** None. **A. Rees:** None. **M.A. Whittington:** None. **F.E.N. LeBeau:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

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Program#/Poster#: 234.13/H17

Topic: B.09. Network Interactions

Support: NIH Grant RR025786

NIH Grant GM103526

Title: Orchestrating the resting brain: Coupling of fast and slow rhythms bypassing alpha

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Abstract: Brain oscillations play an important role in the formation of dynamic functional networks involved in information processing. Specific interaction of brain rhythms, or cross-frequency coupling, is observed in sensory processing and memory functions. FMRI-based functional connectivity analysis in the resting state has become a standard tool to study functional networks and their interaction. However, neuronal signatures of the resting state remain largely unknown. We used multimodal brain imaging based on high density EEG in combination with near-infrared spectroscopy (NIRS) to study cross-frequency coupling on a global scale from infra-slow (~0.1 Hz) to fast (~100 Hz) brain rhythms. EEG (Electrical Geodesics, Eugene, OR) and NIRS signals (two bilateral probes covering inferior and middle frontal gyri; CW5, TechEn, Milford, MA) were recorded at rest (4-8 min) in 15 healthy

volunteers. Broadband EEG (0.1-100 Hz) and optical signals (filtered within 0.01-0.1 Hz to target slow spontaneous oscillations of the resting state networks) were acquired. FFT-based time-frequency analysis was performed at frequencies 0.1-100 Hz for EEG and 0.1 Hz for NIRS. Spectrograms were averaged within 9 frequency bands (slow3, slow2, slow1, delta, theta, alpha, beta, low gamma and high gamma) and their time courses were correlated pairwise. Significant temporal correlation between gamma-band (30-100 Hz) and low frequency modulations (preferentially slow1 and delta bands 0.6-4 Hz) was observed in EEG of all subjects. This correlation was highest in the frontal midline electrodes with weaker secondary maxima in the central parietal and inferior frontal-temporal electrodes. The lowest correlation was observed between gamma and alpha activity. Of all EEG frequencies, gamma power modulations showed the highest correlation with infra-slow hemodynamic modulations at 0.1 Hz while alpha activity showed the lowest correlation with NIRS. These results extend previous observations on the correlation of gamma activity with slower rhythms (mostly theta) during specific cognitive tasks. In the resting state, which is distinct from both cognitive processing and slow-wave sleep, gamma-band activity is coupled with slow rhythms 0.6-4 Hz. Interestingly, gamma power modulations also showed the best correlation with hemodynamic activity. Overall, these results demonstrate global coupling between high and low frequency rhythms of the brain including slow hemodynamic processes. This suggests that such coupling is indicative of the cooperation within the global hierarchy of brain rhythms spanning several levels of organization from cellular to neural network to regional vascular.

Disclosures: A.V. Medvedev: None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

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Topic: B.09. Network Interactions

Support: This work has been supported by grants from the German Research Foundation (SFB 936/A2).

Title: Dose-dependent effects of isoflurane on large-scale intra-cortical communication in the ferret

Authors: F. FISCHER, *F. PIEPER, E. GALINDO-LEON, G. ENGLER, A. K. ENGEL; Dept. of Neurophysiol. and Pathophysiology, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Functional connectivity of brain regions is altered by the administration of anesthetics, leading to the intended reversible loss of perception of external stimuli during surgery. However, the emergence of altered connectivity patterns during gradual change of the anesthesia is not yet well understood. Here, we have investigated connectivity changes in anesthetized ferrets during step-wise increase and decrease of isoflurane concentration. We recorded the spontaneous cortical activity using a custom-made 64-channel subdural electrode array (ECoG) adapted to the ferret's left parieto-temporo-occipital cortex (N=5). The recording sites had a diameter of 250µm and were equally spaced at 1.5mm distance. LFP-like data were filtered between 0.1 Hz and 357 Hz, digitized and down-sampled to 1.4 kHz. We systematically varied the concentration of ventilated isoflurane between 0.4% and 1.6% in steps of 0.2%, and the MAC values were recorded. At each step the concentration was kept constant for 20 minutes to allow equilibration of a stable network state. The last three minutes of this epoch were chosen for analysis. All recordings were performed in a sound- and light-attenuated chamber. Data analysis was performed with Matlab, partly using the 'fieldtrip'-toolbox. We show that the spectral composition of the region-specific (primary visual, parietal and primary auditory) cortical activity changes as a function of the isoflurane concentration. Especially at low frequencies around 0.1Hz we find small correlated and phase-locked cortical patches that show a strong, systematic increase in size with increasing isoflurane concentration up to 1.2%. Further increase of the isoflurane concentration, however, yields a breakdown of the large phase-stable patches and distinct groups of spatially separated but synchronized recording sites emerge. For frequencies between ~1Hz up to 128Hz, overall power-correlations gradually increase across the spectrum as a function of the isoflurane concentration with some specific peaks at ~1Hz and ~3.5Hz. However, within primary visual and auditory areas we observe lower synchronization in the delta-, theta-, and alpha-band compared to higher brain regions. Our data suggest that network states vary with the dose of isoflurane, causing intrinsic changes in cortico-cortical information routing. These findings may be important for better understanding the neural mechanisms leading to loss of consciousness under anesthesia.

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Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.15/H19

Topic: B.09. Network Interactions

Title: Electrophysiological correlates of cortical programs during planned and unplanned terminations of movements in humans with different levels of impulsivity and hyperactivity

Authors: *A. B. TREMBACH, D. SAMARSKIY, V. TOLOKONIKOVA, T. PONOMAREVA, O. KLEIMENOVA;
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Abstract: Our studies showed that hyperactivity and impulsivity are the predominant symptoms in adults with Attention-Deficit/Hyperactivity Disorder (ADHD). It may be caused by disturbances in inhibition system during realization of termination of motions. However, its electrophysiological correlates are not clear. Purpose of the study was to analyze dynamics of EEG during planned and unplanned terminations of movements in healthy and ADHD patients. EEG was recorded in the two groups: healthy (23) and ADHD (25) subjects during resting state, initiation, realization and termination of voluntary movement (M.Adduction policis). Termination of movement was analyzed in two experimental conditions: planned voluntary movement on their own accord and unplanned - the prohibition on the movement in the paradigm of GO/NOGO. The Grand-average topographic maps of EEG spectrum power (EEGSP) and inter- and intra- hemispheric EEG spectrum power coherence (EEGCoh) in frequency band 4-47 Hz were compared in each group between experimental situations. TMS was applied for recording motor evoked potentials (MEP) of M. Adduction pollicis for detecting excitability of primary motor cortex during rest state and termination of movement. Healthy subjects. Planned termination of movement is accompanied by improved EEGSP across the cortical surface in the band of 8-10 Hz and in frontal areas in the bands of 25-35, 36-47 Hz. EEGCoh predominantly increased within these ranges between front and parietal areas of the cortex. Unplanned termination of movement was accompanied by improved EEGSP, EEGCoh in frontal areas in band 11-13 Hz and right hemisphere in the band of 14-24 Hz. Amplitude of MEP of M. Adduction pollicis decreased from 2,1 to 1,1mV. ADHD patients. During planned termination of movement increased EEGSP, EEGCoh was detected all surface of the cortex in the band of 4-7, 8-10 Hz, in the central areas of the cortex - in band 11-13, 14-24, 25-35 Hz. However EEGCoh these frequency bands decreased. During realization of unplanned termination of movements EEGSP increased in the left hemisphere in the band of 8-10, 14-24 Hz and in the parietal and occipital regions of the cortex in the band of 11-13 Hz. . Amplitude of MEP had not changed. Increase of cortical activity in most the frequency bands for the planned termination of motion and its reducing in the frontal areas, constancy excitability of the motor cortex in patients with ADHD compared with healthy subjects may be due to disruption of mechanism the inhibition of motor acts.

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Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.16/H20

Topic: F.01. Human Cognition and Behavior

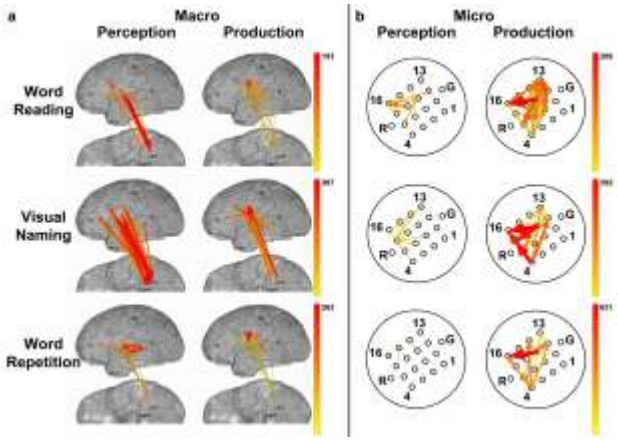
Support: NINDS R01 NS40596

Title: Effective connectivity mapping in humans using hybrid electrocorticographic recordings

Authors: Y. WANG[1], *A. KORZENIEWSKA[2], F. HIGGEN[4], N. E. CRONE[3];
[1]Biomed. Engin., [3]Neurol., [2]Johns Hopkins Univ., Baltimore, MD; [4]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Electrocorticographic signals reflect cognitive processes of underlying neural networks with unique temporal resolution. Newly introduced hybrid electrodes combining macro with microelectrodes provide high spatial resolution. Therefore, task-related functional cortical interactions can be mapped with finer details. We used hybrid electrodes to investigate the dynamics of high-gamma (70-120 Hz) effective connectivity during word reading, visual naming, and word repetition tasks of different perceptual modalities and speech production pathways using event-related causality (ERC). Patterns of causal flows among large-scale networks revealed stimulus- and response-dependent dynamics in the functional topography of eloquent cortex (Fig 1a). During perceptual processing, word reading and visual naming showed bi-directional flows between basal temporal (visual) cortex and Broca's area, while word repetition showed flows from superior temporal gyrus (STG, auditory) to Broca's. In processing related to spoken responses, visual naming, which required word retrieval, showed flows from Broca's to tongue motor area and interactions between motor and basal temporal cortices, while word reading and repetition showed mostly flows among tongue motor areas with weak flows from motor areas to STG. Patterns of flows among local networks within a microelectrode array over ventral postcentral gyrus (Fig. 1b) showed similar differences in relation to spoken responses. The biggest macroelectrode flow (Fig. 1a) was across the micro array, consistent with the array's location in tongue motor area. Among the tasks the most prominent flows occurred during visual naming. The largest flow occurred consistently between the same pair of electrodes in relation to spoken responses during all tasks. To sum up, microelectrode recordings let us observe patterns of effective connectivity among local networks and demonstrate variability in

cortical interactions that are consistent with macro-scale recordings.



Disclosures: Y. Wang: None. **N.E. Crone:** None. **A. Korzeniewska:** None. **F. Higgen:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.17/H21

Topic: B.09. Network Interactions

Support: Clinical and Translational Science Institute of Southeast Wisconsin

Ralph and Marian Falk Medical Research Trust

Departments of Neurology and Neurosurgery at the Medical College of Wisconsin

Title: Quantifying the effects of transcranial magnetic stimulation via computational modeling & electrophysiology

Authors: *B. D. GOODWIN[1], C. R. BUTSON[2];
[1]Biomed. Engin., Marquette, Milwaukee, WI; [2]Neurol. and Neurosurg., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Transcranial magnetic stimulation (TMS) is a method to non-invasively modulate neural activity in the brain. During TMS, a coil is held to the head and energized by the discharge of a capacitor bank; the resulting magnetic field induces current flow in cortical tissue. TMS has been used for investigating the pathophysiology of movement disorders, as well as treating conditions such as depression, blepharospasm, and tinnitus. However, we currently lack a way to quickly and accurately predict the effects of TMS. Such information is critical for

quantifying its effects and gaining a more detailed understanding of the interactions between brain tissue and imposed magnetic field. Computational models have been shown to be a useful and accurate way to predict electromagnetic field interactions within the brain incurred by TMS. Two ongoing challenges in these models are their adaptability to individual subjects, and their ability to predict neural activation. Our objectives were to develop a subject-specific finite element model for TMS that provides the following capabilities: 1) predicts electromagnetic fields for a wide range of coil orientations and tissue properties using a single finite element mesh; 2) predicts neural activation that results from the applied electromagnetic fields. We use electroencephalography (EEG) and electromyography (EMG) to corroborate model predictions on a subject-specific basis. We used computational models combined with electrophysiology to predict neural activation in the hand area of motor cortex during TMS. We designed a coil model based on x-ray measurements (Salinas F, et al., 2007) and a subject-specific finite element mesh to predict electromagnetic fields for arbitrary TMS coil positions and orientations. We then used individual biophysically based pyramidal cell models placed in cortex to quantify the neural activation that occurs during stimulation of the hand knob of motor cortex (M1). A navigation system was used to record coil position/orientation; EMG and 128-channel EEG were recorded while simultaneously administering TMS to the hand-knob in M1. EMG was used to measure motor response magnitude while EEG was used for source localization. An independent component analysis algorithm was used to remove TMS signal artifacts from the EEG. Preliminary results indicate that motor response is acutely sensitive to coil position and orientation, and that physiological recordings can be integrated with computational models of TMS to predict and characterize the sensitivity of the neural response.

Disclosures: B.D. Goodwin: None. **C.R. Butson:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.18/H22

Topic: B.09. Network Interactions

Title: Synchronized brain activity rebound during REM sleep after selective REM sleep deprivation in man

Authors: R. SIFUENTES-ORTEGA[1], Y. DEL RÍO-PORTILLA[1], A. ROSALES-LAGARDE[2], O. A. ROJAS-RAMOS[1], *M. CORSI-CABRERA[1];
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Abstract: Synchrony of fast activity has been postulated to be a common language of the brain involved in processing and integrating information. Converging evidence from neuroimaging and EEG has demonstrated that synchrony/temporal coupling of brain activity changes during rapid eye movement (REM) sleep in comparison to waking and non-REM sleep, increasing between the two hemispheres and decreasing between frontal and posterior association areas. Total sleep deprivation causes homeostatic responses during subsequent recovery sleep; however, total sleep deprivation suppresses both REM and non-REM sleep and homeostatic response of REM cortical synchrony after selective REM sleep deprivation has not yet been studied.

The aim of the present study was to explore the effects of selective REM sleep deprivation (REM-D) on cortical temporal coupling of gamma activity during recovery REM sleep using EEG recordings (19 derivations, 1024 sampling rate). Interhemispheric and intrahemispheric EEG correlations (30-50 Hz) were obtained for the first 3 REM episodes (including phasic events) and for segments without eye movements (tonic REM sleep) during baseline and during recovery nights after one night of selective REM sleep deprivation.

Nineteen healthy subjects were randomly assigned to either selective REM-D, by awakening them at each REM onset, or non-REM interruptions as control for non-specific effects. Subjects slept 4 consecutive nights in the laboratory, the first for habituation, second as baseline, third for sleep manipulation and fourth for recovery.

Interhemispheric temporal coupling and fronto-posterior association areas of the left hemisphere showed a significant homeostatic rebound: interhemispheric temporal coupling increased, while fronto-posterior association areas decreased after REM sleep deprivation.

Present results indicate that after REM sleep deprivation there is a rebound in the increased coupling between the two hemispheres and uncoupling between frontal and posterior association areas normally occurring during REM sleep and suggest that cortical coupling/uncoupling of cortical activity during REM sleep has functional consequences.

In conclusion, these results support the hypothesis that synchronized activity during REM sleep may play an important role in integrating and reprocessing information.

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Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.19/H23

Topic: B.09. Network Interactions

Title: Acute role of corpus callosum on cortical synchrony in humans: Intraoperative electrocorticographic recording before and after callosotomy

Authors: *O. A. ROJAS RAMOS[1], R. ONDARZA[2], J. RAMOS-LOYO[3], I. Y. DEL RÍO-PORTILLA[1], D. TREJO-MARTÍNEZ[4], M. A. GUEVARA[3], M. CORSI-CABRERA[1]; [1]UNAM, Mexico City, Mexico; [2]Inst. Nacional de Neurología y Neurocirugía MVS, Mexico City, Mexico; [3]Inst. de Neurociencias, Univ. de Guadalajara, Guadalajara, Mexico; [4]Hosp. Gen. de México Dr. Eduardo Liceaga, Mexico City, Mexico

Abstract: Synchronization of neural activity between cortical regions is a basic mechanism for integrating information. The use of intraoperative electrocorticography (ECoG) during surgery in patients undergoing callosotomy due to intractable epilepsy offers a unique opportunity to investigate -with greater spatial resolution but no additional surgical intervention- the role of the corpus callosum in cortical synchrony/temporal coupling while avoiding long-term functional reorganization effects.
To investigate the acute role of the corpus callosum on inter and intrahemispheric coherent activity ECoG was recorded during surgery in three patients, in accordance with the Helsinki Declaration, immediately before and after anterior two-thirds callosal transection.
Bilateral electrode grids were placed over frontal cortex and ECoG was recorded and digitized at a sampling rate of 512 Hz, inspected for artifacts and analyzed offline. Spectral power and cross-correlation between inter- and intrahemispheric electrode pairs were obtained for 1 Hz bins and broad bands for each patient immediately before and after callosotomy.
Intrahemispheric temporal coupling decreased significantly in almost all electrode pairs after callosal transaction in the three patients, whereas interhemispheric correlations values were generally very low and did not show significant differences.
In conclusion, interrupting the influence of the corpus callosum acutely affects activity of the contralateral hemisphere by decreasing intrahemispheric temporal coupling. The present results indicate that intrahemispheric temporal coupling does not depend exclusively on ipsilateral cortico-cortical pathways or on subcortical influences, but also on callosal pathways, and suggest that callosal connections play a role in local activity within each hemisphere.
This work constitutes part of an academic thesis of the Psychology PhD program at UNAM.

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Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

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Program#/Poster#: 234.20/H24

Topic: B.09. Network Interactions

Support: NIH Grant 1 R21 DA031853-01A1

Title: Considerations regarding the interpretation of standardized low resolution brain electromagnetic tomography source analysis

Authors: *J. A. CORTES, J. D. CAHILL, M. RANGANATHAN, R. A. SEWELL, D. C. D'SOUZA, P. D. SKOSNIK;
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Abstract: Standardized low resolution electromagnetic tomography (sLORETA) is one of the most popular linear source analysis techniques for the electro and magnetoencephalogram. In this technique, the solution space (e.g. the cortex) is divided in a number of voxels, each of which is assigned a three dimensional vector representing the magnitude and direction of the local current density flow. It's a common practice to convert each current density vector (CDV) into a single positive number consisting in the vector's absolute value. This step facilitates the graphical representation of the activity (one value per voxel) and the statistical analyses. However, it introduces a number of problems that need to be explored and characterized. First, the neural distribution of current density absolute values (CDAV) is no longer a reconstruction of the neural generators underlying the activity recorded at the scalp sensors. Instead, it is a representation of the strength or magnitude at each voxel of the neural activity that co-occurs with the signals recorded at the scalp. CDAVs lack the information about the direction or the polarity (depending on whether the vector's direction is fixed for each voxel) of the CDVs that allow linking the source reconstruction with the observed distribution of activity at the scalp sensors. Thus, CDAVs may increase the risk of considering as a neural generator of some specific scalp response (e.g. the P300
ERP) the activity of voxels not directly involved in it.
Second, it makes the statistical comparisons between groups at the voxel level a comparison between positive scalars. Therefore, the magnitude of the difference between voxels whose vectors change their directions or polarities in the different groups may be greatly reduced. Thus, significant differences between the neural generators underlying the scalp responses associated with different groups may pass
unnoticed.
Third, the inter-subject variability associated to the changes in the directions or polarities of the CDVs of each single voxel may become artificially decreased. Therefore, the comparison of the absolute values of the neural generators underlying the scalp responses of different groups may incorrectly and unpredictably increase or decrease the statistical significance of the differences between them.
To explore these concerns we conducted source analyses on realistic simulations of scalp EEG

recordings. The CDVs and CDVAs were computed and compared. The results of the simulations supported our concerns, suggesting that some of the conclusions that have been previously drawn on the basis of the CDVs may need to be revised.

Disclosures: J.A. Cortes: None. **J.D. Cahill:** None. **M. Ranganathan:** None. **R.A. Sewell:** None. **D.C. D'Souza:** None. **P.D. Skosnik:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

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Program#/Poster#: 234.21/H25

Topic: B.09. Network Interactions

Support: NIH R01-12468

Title: Knowledge-based query and classification of event-related potentials: The neural electromagnetic ontology (nemo) portal

Authors: *N. A. DUNN[1], G. FRISHKOFF[3,2], R. M. FRANK[2];
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[3]Psychology, Georgia State Univ., Atlanta, GA

Abstract: We introduce the Neural ElectroMagnetic Ontology (NEMO) Portal, a web application for classification and storage of event-related potential (ERP) data. The data are represented in Resource Data Format (RDF) files and are automatically generated by the NEMO analysis workflow. These files encode metrics (e.g., peak latency) for each ERP pattern in a dataset. Each pattern is represented as an instance of the superclass ERP_scalp_recorded_component (NEMO-0000093), and is linked to the metrics through assertions such as "Pattern 001 has_peak_latency 120 ms." Specific patterns have defining rules within the ontology, which are used for pattern classification. For example, the N100 is defined as a subclass of NEMO-0000093 that "is maximal over occipital sensors 140-220 ms after onset of a visual stimulus." Thus, if a pattern instance has these properties, a reasoner can infer that it belongs to the N100 pattern class. Previously, we performed inferences in Protégé and would then upload results to the Portal. The Portal now runs inside a Java Virtual Machine, which allows direct integration with the OWLAPI. Thus, data are now classified and labeled automatically when users upload their RDF files. Additions to the graphical interface make it easy to query and retrieve labeled patterns from different datasets and to display their corresponding waveforms and topographic maps (Fig. 1). This means that we can now process and view multiple datasets using an integrated, online application. This represents a major step

towards our goal of providing support for high-quality meta-analysis of ERP data.
[1] G. Frishkoff, J. Sydes, et al., "Minimal Information for Neural Electromagnetic Ontologies (MINEMO): A standards-compliant method for analysis and integration of event-related potentials (ERP) data," Stand Genomic Sci, vol. 5, pp. 211-23, Nov 30 2011.
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Disclosures: N.A. Dunn: None. **G. Frishkoff:** None. **R.M. Frank:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.22/H26

Topic: B.09. Network Interactions

Support: FAU Seed Grant 150605

Title: Atypical patterns of EEG activity and functional connectivity in children with autism

Authors: *N. N. LUCAS, M. PINEDA, N. AARON JONES;
Psychology, Florida Atlantic Univ., Boca Raton, FL

Abstract: Autism is a neurodevelopmental disorder characterized by deficits involving social interaction, communication, and perception. Prevalence estimates have dramatically increased with the current prevalence estimated at one in 88 births. Studies using electroencephalogram (EEG) have shown reduced functional connectivity throughout the brains of autistic individuals, particularly in the beta, delta, and theta frequency bands. In addition, increases in theta power and decreases in alpha and beta power have been reported. Anomalies in EEG coherence and power have been associated with deficits in executive function and mental activity. Although there is much research that has examined EEG activity in individuals with autism, none have examined very young children while awake using EEG power and coherence measures. The present study examined neural activation in children ages 3 -5, during an eyes-closed baseline period. During data collection, children were seated in a chair and fitted with a 14-lead EEG cap with electrodes positioned according to the international 10-20 system. A baseline recording of 3 minutes was collected during quiet wakefulness. Discrete Fourier Transform was performed on artifact-free segments of EEG data to produce power density values. Relative power was examined in the frontal (F3, F4, F7, & F8), temporal (T3, T4, T5, & T6), central (C3 & C4), and occipital (O1 & O2) brain regions. Coherence measurements were examined between the frontal,

temporal, and central region electrodes, as well as between the fronto-temporal regions and between occipital and frontal regions to assess functional connectivity in the alpha bandwidth during the baseline recording. The EEG coherence measurements between channels were obtained by using the complex conjugates of the Fourier coefficients and calculating the average cross spectrum. The value was then squared and normalized by the average residual power spectrum to yield the coherence statistic. Children with autism demonstrated reduced alpha coherence in fronto-temporal regions and between right temporal sites when compared to typically developing (TD) children. In addition, the reduction in coherence was based on autism severity, such that high-functioning children with autism showed greater coherence than low-functioning children with autism. Children with autism also displayed reduced power in the alpha, beta, and delta frequency bandwidths in frontal, temporal, central, and occipital regions compared to TD children. Thus, children with autism exhibited abnormal patterns of brain activity and functional connectivity compared to their typically developing counterparts.

Disclosures: N.N. Lucas: None. **M. Pineda:** None. **N. Aaron Jones:** None.

Poster

234. **Oscillations and Synchrony: EEG and LFP Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 234.23/H27

Topic: B.09. Network Interactions

Support: DFG SFB/TRR 31

DFG Ra 2357/1-1

Title: Efficacy of tACS depends on endogenous oscillatory power

Authors: T. NEULING[1], S. RACH[1], *C. S. HERRMANN[2];
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Abstract: Transcranial alternating current stimulation (tACS) is able to modulate brain oscillations in a frequency specific manner. This offers the possibility to demonstrate a causal relationship between brain oscillations and behavior. Furthermore, tACS is a strong candidate as a tool for clinical applications, however, to fulfill this potential, certain parameters have yet to be evaluated. First, little is known about long-lasting after-effects of tACS with respect to the modulations of rhythmic brain activity. Second, the power of endogenous brain oscillations might play a crucial role in the efficacy of tACS. We hypothesize that the after-effects of tACS depend on the endogenous power of oscillations. To this end, we modulated the power of

endogenous occipital alpha oscillations via tACS. In two experiments, participants either had their eyes open or closed to keep endogenous alpha power either low or high while they were stimulated for 20 min with their individual alpha frequency (IAF) and simultaneously performing a vigilance task. After-effects on IAF power were evaluated over a course of 30 min with a pre stimulation period serving as baseline. After-effects were strongly dependent on IAF power. Enhanced IAF power was observed for at least 30 min after tACS under conditions of low endogenous IAF power, whereas, IAF power could not be further enhanced by tACS under conditions of high IAF power. The current study demonstrates, for the first time, a long lasting effect after tACS on endogenous EEG power in the range of the stimulation frequency. Additionally, we present conclusive evidence that the power of the endogenous oscillations has a critical impact on tACS efficacy. Long lasting after-effects foster the role of tACS as a tool for non-invasive brain stimulation and demonstrate the potential for therapeutic application to reestablish the balance of altered brain oscillations.

Disclosures: T. Neuling: None. **C.S. Herrmann:** None. **S. Rach:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.01/H28

Topic: B.09. Network Interactions

Title: The decoding of emotional information: Insights from intracranial recordings in humans

Authors: *A. CHRISTEN[1,2], L. TAMARIT[2], L. SPINELLI[3], M. SEECK[3], D. GRANDJEAN[1,2]; [1]Univ. of Geneva, Geneva, Switzerland; [2]Swiss Ctr. for Affective Sci., Geneva, Switzerland; [3]Dept. of Neurol., Hôpitaux Universitaires Genevois, Geneva, Switzerland

Abstract: The decoding and the orientation of attention towards emotional information is a central ability that ensure efficient social functioning and behavioral adjustments in our daily life. Although functional neuroimaging data reveal that the amygdala and the orbitofrontal cortex (OFC) are strongly involved in emotion perception, their underlying neuronal dynamics and functional connectivity have not yet been directly investigated in humans. We addressed this issue by recording local field potentials (LFPs) from two epileptic patients who had intracranial macro-electrodes implanted along a mediolateral axis in their temporal and orbitofrontal regions, targeting both amygdalae and the right OFC. In order to manipulate voluntary attention orthogonally to emotional prosody, we used a dichotic listening paradigm, in which the patients were asked to pay attention to one ear in order to perform a gender decision task, while two

sounds were delivered simultaneously (one in each ear for each trial) and appeared in a pseudorandom order. The pseudo-words were presented in both ears, being either neutral on both sides (neutral/neutral), or angry on one side and neutral on the other (anger/neutral or neutral/anger). Our results show that the processing of auditory angry stimuli enhances the power of low-frequency bands and entrains selective, early, and sustained amygdalo-medial OFC synchronization in theta and alpha rhythms. Furthermore, attentional modulations on emotion processing increase high-oscillatory activity (beta and gamma bands) within both regions, although the amygdalae and the medial OFC are more sensitive to the processing of unattended and attended anger stimuli, respectively. Moreover, early and later long-range theta phase coupling in amygdalo-medial OFC regions is augmented during reflexive shifts of attention towards anger stimuli. We propose that the detection and perception of incoming emotional information is achieved through rapid, sustained, and long-range functional amygdalo- medial OFC coupling in low-frequency bands.

Disclosures: A. Christen: None. **L. Tamarit:** None. **L. Spinelli:** None. **M. Seeck:** None. **D. Grandjean:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.02/H29

Topic: B.09. Network Interactions

Support: RO1MH57886

Title: Excess gamma-band activity in schizophrenia interferes with auditory cortical processing

Authors: *L. HAYRYNEN[1], J. P. HAMM[1], S. R. SPONHEIM[2,3], B. A. CLEMENTZ[1]; [1]Univ. of Georgia, Athens, GA; [2]Minneapolis VA Hlth. Care Syst., Minneapolis, MN; [3]Univ. of Minnesota, Minneapolis, MN

Abstract: Low-gamma band cortical activity and prolonged entrainment to 40 Hz auditory steady state stimuli is augmented in a subset of schizophrenia patients (SZ) and in animals administered NMDA antagonists (e.g. ketamine), possibly indicating in both cases disruptions of signal-to-noise ratio in cortical circuits due to imbalances in GABA/glutamate controlled neuronal activity. The current study sought to further understand how these gamma-band augmentations relate to SZ related abnormalities in basic and complex sensory processing in auditory cortices. 248-sensor magneto-encephalography data were collected for 15 SZ and 15 H during an auditory oddball paradigm (25% targets; 1 sec ISI). Auditory stimuli (pure tones: 1

kHz standards; 2 kHz targets) were administered during 4 continuous background (auditory steady state) stimulation conditions: (1) no stimulation; (2) 24 Hz; (3) 40 Hz; (4) 88 Hz. We quantified the disruption of the auditory steady state response (aSSR). This manipulation allowed a measurement of whether enhanced ongoing gamma entrainment in SZ, and related gamma-band augmentations, came at the cost of salient stimulus processing. A Fast Fourier Transform (FFT) was performed in 300ms steps (starting 300ms prestimulus) on continuous single trial data to index the oscillatory power at the driving frequencies of interest (24, 40, 88Hz). Ten peak sensors (five per hemisphere) were chosen based on signal strength and topography. A repeated measures ANOVA was then performed with one between-subjects factor (Diagnosis: SZ, H) and two within-subjects factors (Time: prestimulus, first, second, third 300ms bins; Stimulus: targets, standards) for each steady state stimulation condition. Results indicate a that SZ have accentuated aSSR entrainment at 40 Hz, but not 88 Hz, stimulation conditions compared to H, consistent with reports of sustained gamma-band augmentation seen in NMDA-hypofunction models of psychosis. Additionally, SZ have a significant decrease in power of the ongoing 40Hz steady-state entrainment at the onset of the oddball stimuli (targets and standards). This finding indicates that augmented aSSRs reflect SZ but not H dependence on local (low gamma range) processing to identify stimuli, thus being more dependent on the physical properties of the stimulus.

Disclosures: L. Hayrynen: None. **J.P. Hamm:** None. **B.A. Clementz:** None. **S.R. Sponheim:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.03/H30

Topic: B.09. Network Interactions

Support: NSF GRFP

Title: Population coding with higher order correlations

Authors: *N. CAYCO GAJIC[1], J. ZYLBERBERG[2], E. SHEA-BROWN[2];
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Abstract: Higher-order correlations (beyond pairwise spiking statistics) have been observed in the brain and have been shown to affect coding facility. In the case of pairwise correlations, having signal and noise correlations of the opposite sign will cancel redundancies and allow for better coding; however, no such general rules have yet been investigated for higher-order

statistics.
To understand the effects that higher-order correlations may have on coding capacity in neural populations, we take the approach of assuming that the stimulus-conditioned lower-order statistics - that is, mean firing rates and pairwise noise correlations - are fixed. What is the range of effects that triplet correlations may have on two-stimulus discrimination between given noisy population responses whose lower moments are constrained, e.g. by experiment? For fixed first and second moments, we sweep over possible third-order cumulants by fitting the third-order maximum entropy distribution using the Improved Iterative Scaling algorithm for small networks (up to N = 10 due to computational time). Then, for all possible third-order cumulants under each stimulus, we calculate the mutual information between the two stimuli and the response distributions. We find that, as long as pairwise noise correlations have realistic (small) values, the optimal solution is to increase triplet spiking for the stimulus that induces lower rates, and to decrease triplet spiking for the stimulus that induces higher rates.
How can such higher-order correlations be realized? Recent work suggests that intrinsic nonlinearities in single neurons contribute to such higher-order correlations at the population level: we explore the role of dendritic nonlinearities. First, we interpret our optimized statistical model in light of the of data of Polsky et al., assigning a functional role for their finding that two small inputs to the same dendritic branch are summed superlinearly whereas larger inputs to the same branch should be summed sublinearly. We investigate the viability of this mechanism to produce beneficial higher-order correlations in a balanced network of integrate-and-fire neurons, computing the information that network responses have about its inputs both with and without dendritic nonlinearities.

Disclosures: N. Cayco Gajic: None. **J. Zylberberg:** None. **E. Shea-Brown:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

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Program#/Poster#: 235.04/H31

Topic: B.09. Network Interactions

Support: Center for Alzheimer's Disease and Related Disorders

Title: Refractory periods following spontaneous ripples create opportunities for cortical feedback

Authors: *D. J. KANAK[1,2], G. M. ROSE[1,2], P. R. PATRYLO[1,2];
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Abstract: The sharp wave ripple complex (SWR) is a network pattern generated in the CA3 subregion of the hippocampus (HC) that is believed to facilitate memory consolidation by coordinating the reactivation and transfer of information to the neocortex (NC). While SWRs can arise independently in CA3, their initiation may be biased by neocortical activity. Our in vitro slice study examined the capacity of perforant path (PP) synaptic excitation to elicit SWRs from a spontaneously active pattern generator. Low-intensity electrical stimulation (25-50 μA, 0.5 Hz, 100 pulses) evoked monosynaptic field EPSPs in CA3 that were closely followed by precisely-timed evoked SWRs (eSWR). Although field EPSP amplitudes were stable throughout the stimulus train, a subset of stimuli failed to elicit eSWRs, particularly when closely preceded by spontaneous SWRs (sSWR). We designed a near real-time MATLAB/Simulink application to control stimulus timing during the ~250 ms refractory periods that followed sSWRs. Stimulus delay (25, 50, 100, and 200 ms) had a highly significant effect on eSWR incidence ($F_{1.85, 14.84} = 48.74$, $p < 0.001$, RM-ANOVA), with a maximum value of 0.72 (95% CI = [0.61, 0.81]) 200 ms after sSWR onset. In contrast, the estimated incidence of sSWRs was much lower throughout the refractory period, reaching a maximum value of only 0.03 (95% CI = [0.015, 0.049]) at 200 ms. Fixing the stimulus delay at 200 ms, we then compared the network and single cell properties of sSWRs and eSWRs. Both exhibited ripple oscillations of comparable power and frequency, and both were associated with similar temporal patterning of single unit activity. Stimulating the medial entorhinal cortex (MEC) could also elicit eSWRs, and lesions targeting the direct PP input to CA3 while sparing the DG substantially reduced eSWR incidence. In summary, direct MEC input to CA3 can initiate SWRs at times when self-organizing mechanisms generally cannot. Assuming sSWRs convey information to the NC, the ensuing refractory periods might allow opportunities for cortical feedback to reinforce the recently engaged cell assembly. In the absence of such feedback, CA3 could then resume the quasi-random process of self-organizing the reactivation of its memory content.

Disclosures: D.J. Kanak: None. **G.M. Rose:** None. **P.R. Patrylo:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.05/H32

Topic: B.09. Network Interactions

Title: Functionally stable hippocampal network: Role of inhibition vs. short-term plasticity

Authors: *A. M. HUMMOS, S. NAIR;
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Abstract: The associational recurrent connections in the hippocamapal CA3 area support specialized hippocampal functions from retrieval of memories given partial cues to replaying temporal sequences of memories. Nevertheless, the same property renders the network inherently unstable and susceptible to runway excitation and synchronized aberrant discharges. Such instability poses serious problems in building a functioning hippocampal model while avoiding such catastrophic dynamics.
We developed a biophysical model of entorhinal cortex (EC), dentate gyrus (DG), and region CA3 using biological data and known recordings at multiple levels. We match cell behaviors, kinetics of synaptic currents, short-term and long-term synaptic plasticity, and network connectivity and 3D organization. The model's functionality and validity were assessed by its ability to perform pattern separation and pattern completion matching experimental recordings from DG and CA3. Finally, candidate stability mechanisms used in constructing the model were further evaluated.
Adding the inhibitory basket cells (BC) to the model improved stability, but at the expense of the model's ability to form stable representations. In contrast, implementing short-term synaptic depression at the CA3 associational/commissural recurrent connections proved successful at enhancing stability while maintaining the functional representations needed for the model to perform its tasks. Importantly, and contrary to our initial hypothesis, adding OLM inhibition did not provide significant advantages in network stability.
Our results indicate a prominent role for short-term synaptic depression at AC fibers in the stability of the network. Additionally, the results also suggest a limited homeostatic role for both BC and OLM interneurons at the studied timescale. Such results challenge the current conceptualization of OLM cells as 'feedback' inhibitory cells and point towards other roles, mainly, generation of theta oscillatory behavior which we investigate in detail.

Disclosures: A.M. Hummos: None. **S. Nair:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

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Topic: B.09. Network Interactions

Support: NINDS: 1F31 NS083247

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Title: Ventral postrhinal cortex: A strong inhibitory network in the absence of parvalbumin-expressing interneurons

Authors: *A. U. SUGDEN, S. L. PATRICK, A. ANAND, R. D. BURWELL, B. W. CONNORS;
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Abstract: Synaptic inhibition in the cerebral cortex is vital for gain control, regulating temporal and spatial features of coding, generating gamma oscillations, and preventing seizures. Inhibitory interneurons in neocortex are diverse. The broadest categories include parvalbumin (PV)-, somatostatin (Som)-, and 5HT3aR-expressing cells; PV cells are the most common class, comprising about 40% of the interneurons in most cortical areas. However, in the ventral postrhinal cortex (vPOR; a parahippocampal region), PV cells are essentially absent. We asked how inhibitory functions are achieved in a cortical circuit that lacks PV interneurons. Interneuron distributions were analyzed using immunohistochemistry and transgenic mice that express fluorescent proteins selectively in interneuron subtypes. The density of PV interneurons was dramatically lower in vPOR than in adjacent regions such as dorsal POR (dPOR). The densities of Som and 5HT3aR interneurons, by contrast, were similar in vPOR and dPOR. Thus, there are fewer interneurons overall in the vPOR. Whole-cell recordings from vPOR pyramidal cells showed that spontaneous IPSCs were similar in amplitude but lower in frequency compared to those of dPOR cells. Surprisingly, when all types of interneurons were activated optically or electrically, inhibitory currents in pyramidal cells of vPOR and adjacent dPOR were indistinguishable. Paired-pulse ratios for electrically evoked IPSCs were similar in both regions of POR. Selective optogenetic activation of PV interneurons triggered IPSCs that were ten-fold larger in cells of the dPOR than in cells of vPOR. This suggests that the absence of PV cell-mediated inhibition in vPOR is compensated for by stronger inhibitory circuits from Som or 5HT3aR cells to maintain normal global levels of inhibition.

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Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.07/H34

Topic: B.09. Network Interactions

Support: UNC School of Medicine

UNC Department of Psychiatry

Title: Transcranial alternating current stimulation (tACS) induces state switching in interconnected cortical networks

Authors: *F. FROHLICH, K. M. KUTCHKO;
Psychiatry, Univ. of North Carolina, Chapel Hill, NC

Abstract: Non-invasive brain stimulation with transcranial current stimulation (TCS) generates a weak electric field that modulates the membrane voltage of cortical neurons. Using sine-wave stimulation waveforms (transcranial alternating current stimulation, tACS) causes enhancement of cortical oscillations that outlasts the application of stimulation. The underlying mechanism of these outlasting effects remains unknown. Understanding the cellular and network level mechanisms by which tACS causes outlasting changes in cortical activity is critical for rational design of stimulation paradigms that optimize the type and duration of outlasting response based on individual therapeutic needs.

We used large-scale computer simulations of two interconnected networks to probe the effects of weak global perturbations generated by weak electric fields on global "internetwork" dynamics. Both networks consisted of a 2D layer of pyramidal cells (PYs, 160,000 Izhikevich model neurons) and a 2D layer of inhibitory interneurons (INs, 40,000 Izhikevich model neurons). PYs were connected by excitatory synapses. PYs also excited INs that inhibited PYs. The local synaptic connectivity was chosen so the networks intrinsically oscillated at 3Hz. Excitatory long-range projections were sparse and exhibited varying delays (up to 50 msec). Introducing these long-range projections with delays caused the emergence of multiple, metastable activity states with qualitatively different spatio-temporal signatures: (1) rapid fire (globally synchronized), (2) slow, propagating planar waves, and (3) spiral waves. The internetwork spontaneously switched between these states with a time-scale of several seconds. Importantly, the application of tACS not only introduced a switch to the rapid fire, synchronized state in the majority of simulations but also had an outlasting effect after offset of tACS. These results suggest that the outlasting effects of tACS may be mediated by the underlying multistability of cortical internetworks. In this framework, tACS acts as a perturbation that induces state transitions. This contrasts with the prevalent view that effects of TCS are mediated by synaptic or intrinsic plasticity.

In summary, computer simulations of the modulation of large-scale brain dynamics by tACS offer (1) new mechanistic understanding of how non-invasive brain stimulation works and (2)

insights for the generation of novel hypotheses about emergent multistable internetwork dynamics that can subsequently be tested in experiments.

Disclosures: F. Frohlich: None. **K.M. Kutchko:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.08/H35

Topic: B.09. Network Interactions

Support: UNC School of Medicine

UNC Department of Psychiatry

Title: Optogenetic dissection of cortical neuromodulation with transcranial current stimulation

Authors: *S. L. SCHMIDT, F. FROHLICH;
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Abstract: Transcranial Current Stimulation (TCS) has shown promise as a treatment for neuropsychiatric disorders and for augmenting cognition. Specifically, TCS with sine-wave stimulation waveforms (transcranial Alternating Current Stimulation, tACS) may augment cortical oscillations in a targeted manner. However, it remains unclear how to choose stimulation frequencies and amplitudes for optimal treatment responses. Such rational design of non-invasive brain stimulation with individualized stimulation frequency requires an understanding of the interaction between intrinsic oscillations and applied stimulation waveform. Our previous modeling results have suggested that network resonance mediates the effects of tACS on cortical oscillations.

Here, we experimentally tested this hypothesis in vitro through the use of optogenetics. We performed multielectrode array (MEA) recordings of multiunit spiking activity in acute cortical slices while applying electric fields that mimicked the effects of TCS. Multiunit activity recorded on 59 channels spaced 200μm apart allowed us to assess responses in all cortical layers. Using Thy1-ChR2 juvenile mice (p15-p30), which express channelrhodopsin in Layer V pyramidal cells, we successfully entrained the network at 1Hz by applying 500ms pulses of light. We then sought to enhance this rhythm by applying an electric field at the entrained frequency. Furthermore, we examined the effect of applying sine-wave electric fields with frequencies differing from the dominant ongoing network oscillation. We hypothesized that the effect strength of tACS is increased when the frequencies of the ongoing network oscillations and the

stimulation are the same. We quantified entrainment both with frequency analysis and with measures of spike timing relative to the phase of stimulation. Using these measures, we found not only that Layer V pyramidal cells oscillate at 1Hz in response to optogenetic stimulation but also that this change propagated through the network, entraining cells in all cortical layers. Such mechanistic study of the interaction of cortical oscillations and sine-wave electric field stimulation provides an important stepping stone towards rational design of non-invasive brain stimulation with optimized, individualized stimulation frequencies. Such improved tACS paradigms may enable the targeted treatment of psychiatric symptoms such as impaired cognition that are associated with deficits in cortical oscillations.

Disclosures: S.L. Schmidt: None. **F. Frohlich:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

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UNC School of Medicine

UNC Department of Psychiatry

Title: Targeting cortical oscillations and cognition with transcranial alternating current stimulation (tACS) in humans

Authors: *M. R. BOYLE, A. L. CORDLE, W. L. GORIS, K. K. SELLERS, B. V. VAUGHN, J. H. GILMORE, F. FROHLICH;
Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Transcranial current stimulation (TCS) has been shown to (1) modulate macroscopic brain activity measured by electroencephalogram (EEG), (2) alter behavioral performance in a range of cognitive tasks, and (3) alleviate symptoms in patients with neuropsychiatric illnesses [1]. Recent studies have indicated that TCS with sine-wave stimulation waveforms (transcranial alternating current stimulation, tACS) may modulate cortical oscillations in a frequency-dependent manner. Given the fundamental role of cortical oscillation in cognition, tACS has become increasingly promising as a potential non-invasive treatment for neuropsychiatric

illnesses with cognitive impairment such as schizophrenia and autism.
However, the evidence for a causal link between patterns of EEG activity and specific behavioral function remains incomplete. We here combined EEG, tACS, and behavioral measures to probe the causal effect of non-invasive brain stimulation on both endogenous cortical oscillations and behavioral performance in a battery of psychophysical tests.
We performed whole-head EEG (10-20 system) on healthy human subjects. In the first study, we compared the effect of active sham, 0.75 Hz, and 40 Hz tACS on cortical oscillations during resting (0.75 mA peak-to-peak). In the second study, subjects performed visual working memory, visual detection, and visual closure tasks in random order without stimulation. Subsequently, subjects received 1 hour sessions of sham stimulation, individualized alpha frequency (IAF) tACS, and 40 Hz tACS in randomized order during which they completed the same tasks. In this study, 2mA peak-to-peak stimulation was applied to target occipitoparietal areas. EEG signals were analyzed with independent component analysis (ICA), extreme value rejection (EVR), and Morlet wavelets to extract temporally resolved frequency content of cortical activity.
We found that tACS significantly modulated cortical oscillations. In particular, 40 Hz tACS enhanced gamma oscillations and concurrently suppressed alpha oscillations. These results suggest that tACS can dynamically interact with the endogenous balance of slow "offline" (resting) and fast "online" cortical rhythms. Our combined psychophysical and brain stimulation study will further elucidate the role of such targeted stimulation in modulating cognitive performance.
1.
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Poster

235. **Network Interactions: Oscillations and Synchrony II**

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Title: Modulations and plasticity of oscillation amplitudes and synchronization in human amblyopia

Authors: H. JULKU[1], S. ROUHINEN[1], J. MÄKELÄ[2], H. HUTTUNEN[1], E. CASTRÉN[1], J. M. PALVA[1], *S. PALVA[1];
[1]Univ. of Helsinki, Neurosci. Ctr., University of Helsinki, Finland; [2]HUS Med. Imaging Ctr., BioMag Lab., Helsinki University Central Hospital, Finland

Abstract: Amblyopia is characterized by poor or indistinct vision in an eye that is physically normal and caused by cortical structural and functional abnormalities. Amblyopic eye has lowered visual acuity, temporal integration, motion detection, and perception of multiple visual objects (1,2,3). Functional magnetic resonance imaging has revealed that these deficits are associated with decreased neuronal activity (4,5) and connectivity (6) in the primary visual cortex but also at higher levels of visual cortical hierarchy. Less is known of the electrophysiological correlates of human amblyopia.
In recent years, several studies have shown that oscillations and synchronization are key features in the coordination of visual processing and in the formation of visual object representations (7). To address whether amblyopia is associated with abnormal oscillation amplitudes and synchronization, we recorded ongoing neuronal activity with magnetoencephalography (MEG) and used minimum-norm-estimate based source reconstruction to map neuronal dynamics in the individual cortical anatomy of each patient. Patients were recruited from a clinical study (sponsored by HermoPharma Ltd) that investigated effects of fluoxetine and / or visual training on treatment of adult anisometric and strabismic amblyopia (0.30-1.10 LogMAR). We recorded MEG during several types of visual attention, perception, and memory tasks before and after the treatment. We estimated strength of oscillation amplitudes, synchronization, and evoked responses separately to the amblyopic and the fellow eye. We found that both oscillation amplitudes and their phase-locking to stimulus onset were stronger for stimuli presented to the fellow than to the amblyopic eye in several levels of visual cortical hierarchy. The extent of these changes was also larger for complex visual stimuli. Importantly, oscillation amplitudes were stronger to stimuli presented to the fellow eye not only during processing of visual information, but also during memory retention period suggesting that the deficits in amblyopia extend to higher-level cognitive processing. In the follow-up analyses we will investigate whether fluoxetine and / or visual training treatment modulate the strength of visual evoked responses, oscillations amplitudes and synchronization.
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Poster

235. **Network Interactions: Oscillations and Synchrony II**

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Topic: B.09. Network Interactions

Support: BrainGain Smart Mix Programme of the Dutch Ministry of Economic Affairs and the Dutch Ministry of Education, Culture and Science

Title: Patterns of oscillatory phase relations in human electrocorticography can be explained by temporal delays

Authors: *R. VAN DER MEIJ[1], J. JACOBS[2], E. MARIS[1];
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Abstract: In a rapidly changing environment the brain needs to transfer information over many neuronal networks quickly and flexibly. One way this could be achieved is through consistent phase relations, where an ideal phase relation corresponds to the time delay at which two neuronal populations are at peak excitability. These populations could be close together and at a distance, involving respectively small and large synaptic delays. These shared windows of communication occur rhythmically, are measurable at various spatial scales, and occur across many frequencies.
If consistent phase relations are used for the routing of information, then such consistency should be spread out over the cortex. Characterizing spatially distributed phase relations presents us with two challenges. Firstly, phase relations between many electrodes at many frequencies cannot be analyzed by simple pair wise analyses, as the full spatial and spectral structure would not be captured. And secondly, under the assumption that synaptic delays between two

communicating neuronal populations are constant, a time delay between two locations would be reflected in phase relations that are a linear function of the involved frequencies.
To meet these challenges, we developed a new decomposition technique, with which we model the linear relation between phase and frequency as a time delay. We analyzed 61 human electrocorticographic recordings of 28 patients scheduled for resective epilepsy surgery, while patients were performing a working memory task. We were able to decompose Fourier coefficients over electrodes, frequencies and trials into sets of components. Each component is described by four loading vectors: (1) a frequency profile, (2) a trial profile, and a spatial map describing (3) the degree of involvement of each electrode and (4) the time delays between the electrodes. Thus, each component describes a spatio-spectral pattern of phase relations and, importantly, the temporal structure of possible information flow in the underlying network. Using the above model we were able to extract patterns at different spatial scales and in different frequency ranges.

Disclosures: R. Van Der Meij: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Radboud University Nijmegen. **J. Jacobs:** None. **E. Maris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Radboud University Nijmegen.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.12/I3

Topic: B.09. Network Interactions

Support: OTKA K81354

OTKA PD77864

ANR-TÉT Neurogen

ANR-TÉT Multisca

FP7 Neuroseeker

TÁMOP-4.2.1.B-11/2/KMR-2011-0002

TÁMOP-4.2.2./B-10/1-2010-0014

Title: Simultaneous Ca-imaging and electrophysiology of human cortical population activity *In vitro*

Authors: *B. P. KEREKES[1,2], A. KASZÁS[3], K. TÓTH[3], B. CHIOVINI[3], G. SZALAY[3], D. PÁLFY[3], K. SPITZER[3], I. ULBERT[2,1], L. WITTNER[2], B. RÓZS[3];
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Abstract: Spontaneous synchronous population activity (SPA) emerges from the cortical slices of epileptic and non-epileptic patients maintained in physiological medium in vitro. SPA was recorded until now using sharp intracellular and laminar extracellular methods to analyze the neural mechanisms giving rise to population synaptic/trans-membrane and spiking activity.
In order to gain additional spatial information about the network mechanisms involved in the SPA generation, we introduced the two-photon Ca-imaging technique on human in vitro slice preparations. The excellent spatial coverage and resolution of this technique supplements the lower spatial resolution but higher temporal resolution of laminar extracellular, sharp intracellular and whole cell patch recording techniques.
Human slices were maintained in a dual superfusion chamber of high flow rate physiological incubation medium and otherwise conventional submerged technique to elicit SPA in a two-photon microscope. The population activity was recorded by laminar extracellular electrodes and with extracellular patch electrode. After identifying the active regions of the slice using electrophysiology techniques, bolus loading of OGB-1 and SR101 was applied on the tissue. The neuronal and glial cells took up these dies, thus we were able to image the SPA related Ca-transients in pyramidal cells with two-photon technique, simultaneously with extracellular and whole cell patch measurements.
Combining high spatial resolution two-photon Ca-imaging technique and high temporal resolution extra- and intracellular electrophysiology techniques may permit a deeper understanding about the network properties of SPA in the human cortex.

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Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.13/I4

Topic: B.09. Network Interactions

Support: CIHR

Title: Theta oscillations link local and interlaminar communication within the Human neocortex

Authors: R. J. MCGINN[1], C. L. FLOREZ[3], V. LUKANKIN[3], J. A. DIAN[1], S. R. SUGUMAR[2], P. L. CARLEN[3], *L. ZHANG[3], T. A. VALIANTE[3];
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Abstract: Objective: We were interested in exploring interlaminar oscillations and their putative role in neocortical communication and computation. Cross frequency coupling (CFC) is suggested to represent a neural code implicated in memory and attention and to reflect local neural computation. On a large scale, inter-regional oscillatory coherence is thought to underlie long-range communication and network formation, the so-called communication through coherence (CTC) hypothesis. We used human neocortical slices to better understand these two paradigmatic theories. In particular, we hypothesized that the microcircuits that underlie these modes of communication likely overlap, and thus local and long range communication should be coordinated. Here we show that indeed strength of CFC and CTC are correlated, suggesting a dynamical link between local and interlaminar communication.
Methods: Temporal neocortical tissue was resected during epilepsy surgery. 500 um slices were placed in artificial cerebral spinal fluid and local field recordings were obtained from superfical and deep cortical layers. Kainate (50 nM) and carbachol (50uM) were used in order to model exogenous inputs. The metrics used were: 1) modulation index, which quantifies how one frequency modulates another, 2) phase coherence (PC) which assesses the temporal synchrony between the two layers, and 3) PDPC, a mixed metric of amplitude relationships between the two layers as a function of temporal relations. These metrics were computed over a 30s time period and correlated using Spearman rank correlation.
Results: Twenty-five slices from ten patients displayed rhythmic activity above baseline conditions. PC between layers indicated that activity in deep layers led that in superficial layers. CFC between theta (4-15 Hz) and gamma (>30 Hz) frequencies and a novel low-frequency PDPC were observed. At theta frequencies, a linear relationship between the frequencies of maximal CFC and maximal PDPC was observed. Furthermore, the values of these metrics were found to be significantly and positively correlated ($p<0.05$). Fitting the PDPC values to an analytic model suggested that this interlaminar communication at theta frequencies was bidirectional.
Conclusions: Here we show a number of novel findings: 1) theta frequency oscillations appear to be generated from deep cortical layers, 2) PDPC between cortical layers is strongest at theta frequencies, and 3) that such theta PDPC is correlated to CFC between theta and gamma activity. We highlight the privileged role that theta oscillations have been suggested to play in long range communication (PDPC), and link this to a metric of local computation (CFC).

Disclosures: R.J. McGinn: None. **C.L. Florez:** None. **V. Lukankin:** None. **J.A. Dian:** None. **S.R. Sugumar:** None. **P.L. Carlen:** None. **L. Zhang:** None. **T.A. Valiante:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

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Program#/Poster#: 235.14/I5

Topic: B.09. Network Interactions

Support: ERANET-Neuron project EPINET (EUI2009-04093) from EU FP7, Spanish Ministry MICINN (BFU2009-07989)

Spanish National Research Council (CSIC 200720I023) to LMP

Title: Specific reorganization of theta-phase related gamma activity in experimental models of temporal lobe epilepsy

Authors: *M. VALDERRAMA[1], J. BROTONS-MAS[2], F. LAURENT[2], L. MENENDEZ DE LA PRIDA[2];
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Abstract: Gamma activities (~ 30-100 Hz) in the hippocampus have been associated with different cognitive processes including the integration of environmental spatio-temporal information, and memory formation and recall. They result from precise circuit interactions that are modulated by the phases of the hippocampal theta waves, especially during active exploration and REM sleep. Here, we study the profile of gamma activities in the hippocampus of epileptic (n=4) and control (n=4) rats performing an object exploration task. In particular, we look for differences of theta-gamma interaction at the hippocampal CA1 region. To this purpose, we recorded local field potentials longwise different strata using multi-site silicon probes in freely moving animals. First, we selected individual theta waves (4-12 Hz) from the stratum lacunosum moleculare that were fulfilling defined amplitude criteria. From these, we selected the corresponding theta waves at the pyramidal layer and considered individual waves centered on the positive peak inside a 1.25 sec window. For each window, we estimated the activity in the entire gamma band through a wavelet transformation. We report here that although gamma activity is properly modulated by theta waves in both, control and epileptic animals, the organization along the 30-100 Hz band is not the same for both groups. In particular, while activities in the epileptic group appeared to be concentrated in the higher gamma band (~ 80 Hz) and absent in intermediate bands (~ 45-70 Hz), oscillations covering the entire gamma range are

present in the control group. The power of oscillations in the high-gamma band is significant higher in epileptics compared to control animals ($p < 0.001$, permutation test). Importantly, similar theta-gamma phase relationship was present between groups, suggesting that similar cellular processes were involved. Taken together, our results suggest a different reorganization of processes involving gamma oscillations in the hippocampus of epileptic animals that could be potentially linked to deficiencies in cognitive or memory functions.

Disclosures: M. Valderrama: None. **J. Brotons-Mas:** None. **F. Laurent:** None. **L. Menendez de la Prida:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.15/I6

Topic: B.09. Network Interactions

Support: Intramural Research Program of the National Institute of Mental Health

Title: Entropy fluctuations in MEG signals above 150 Hz are similar to low frequency oscillations

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[1]MEG Core Group, NIH/NIMH, Bethesda, MD; [2]Multimedia Imaging Lab., Univ. of California San Diego, San Diego, CA

Abstract: Complexity analysis of MEG signals above 150 Hz reveals fluctuations at beta and gamma band frequencies. This may indicate the presence of multi-unit activity (MUA) in the signal. The rank vector entropy (RVE) transform is a broad-band measure of signal complexity and is distinct from measures of power [1]. It is most effective when applied to broadband signals (narrow-band signals have low complexity). MEG signals above 150 Hz generally have poor signal-to-noise ratio and lack distinct spectral peaks, signals. Exceptions are the 600 Hz oscillations coincident with the somatosensory N20 response to median nerve electrical stimulation [2] and the high frequency oscillations (HFOs) and fast ripples in some epilepsy recordings [3]. Functional images of activity in a 150-300 Hz bandpass were imaged using RVE (HF-RVE) from working memory studies of six normal subjects, using a 275-channel MEG instrument (CTF Systems, Inc.). Dorsolateral prefrontal, anterior cingulate, and posterior parietal areas showed significant ($p < 10\text{-}5$) changes between the 2-back and 0-back conditions. In addition to these event-related changes, we observed gamma band oscillations in the time-series from parietal voxels. Oscillations could not be replicated from surrogate data where the sample

order was randomized prior to RVE, indicating that they are hidden property of the high-frequency signals. How do these oscillations in HF-RVE relate to those appearing in a 4 to 150 Hz bandpass (LF)? To test this we analyzed the coherence between the low and RVE-transformed high frequencies. Coherence was small, primarily due to the non-stationary behavior of the oscillations. Tests of mutual information were also performed, yielding larger values. We speculate that the high-frequency signal relates to the regional MUA. If this hypothesis is correct, then it may be possible to discriminate between regional outputs (the HF-RVE) and the inputs to the dendritic tree (LF). Seed based analyses reveal regions with high mutual information relative to the seed. Mutual information was not reciprocal, suggesting directional connectivity.
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2.Fedele, T., et al., Towards non-invasive multi-unit spike recordings: mapping 1kHz EEG signals over human somatosensory cortex. Clin Neurophysiol, 2012. 123(12): p. 2370-6.
3.Worrell, G.A., et al., High-frequency oscillations and seizure generation in neocortical epilepsy. Brain, 2004. 127: p. 1496-1506.

Disclosures: S.E. Robinson: None. **A.J. Mandell:** None. **R. Coppola:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.16/I7

Topic: B.09. Network Interactions

Title: Role of 5HT2 and 5HT3 receptors in the generation of epileptiform activity: *In vitro*, in silico and *In vivo* studies

Authors: *P. A. PUZEREY, R. FERNÁNDEZ GALÁN;
Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: The broad expression of serotonin (5-HT) receptors in the neocortex is juxtaposed with dense innervation by serotonergic afferents, underscoring the influence of 5-HT on cortical activity. While the effects of 5-HT are well understood at the level of the neuron, its role in network activity remains unclear. We have shown that elevating 5-HT in disinhibited cortical slices with the selective serotonin reuptake inhibitor, fluoxetine (FLX), transforms cortical network dynamics from single bursts, known as paroxysmal depolarizing shifts (PDS), to periodic (15 Hz) burst clusters known as paroxysmal fast runs (PFRs), a form of activity often seen in animal and human epileptic seizures. The emergence of PFRs depends on 5-HT2

receptors (5-HT2Rs) as they are blocked by ketanserin (KSN). We set out to investigate the mechanistic underpinnings of the 5HT2R-dependent switch from temporally random and sparse to periodic and clustered network bursts. To this end, we measured postsynaptic currents during network events in control and FLX-treated cortical slices and observed an increase in excitatory and a decrease in the inhibitory postsynaptic currents (EPSCs & IPSCs). KSN reduced the enhanced EPSCs to control levels, while further decreasing IPSCs. We confirmed the sufficiency of these changes to the emergence of PFRs using a computer simulation of a model cortical network. Furthermore, since the emergence of PFRs depends on 5-HT2Rs, we tested in vivo whether 5-HT2Rs modulate epileptic seizures, the behavioral correlate of the PFRs observed in vitro. Indeed, injection of KSN before seizure induction with pentylenetetrazole (PTZ) significantly delayed the onset of epileptic seizures. In parallel with 5-HT2R-dependent modulation of network activity, we also explored the role of 5-HT3 receptors (5-HT3R) in cortical dynamics. We previously showed that FLX enhances spontaneous synaptic activity through 5-HT3Rs in cortical neurons. Blocking 5-HT3Rs with granisetron in FLX-treated disinhibited slices results in a significant reduction of network bursts, though the emergence of PFRs remains unaffected. Combined, our results present a mechanism by which augmented 5-HT signaling in the cortex alters cortical dynamics: 5-HT2Rs act in concert with 5-HT3Rs on cortical neurons to elevate global levels of excitation, while 5-HT2R activity transforms cortical activity patterns from random bursts to highly periodic fast runs of paroxysmal discharges. These findings emphasize the importance of neuromodulatory control in shaping cortical dynamics and provide a potential therapeutic avenue for treating epileptic seizures that are resistant to typical antiepileptic drugs.

Disclosures: P.A. Puzerey: None. **R. Fernández Galán:** None.

Poster

235. **Network Interactions: Oscillations and Synchrony II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 235.17/I8

Topic: B.09. Network Interactions

Title: Effect of coupling frequency on information sharing

Authors: *C. A. STETSON;
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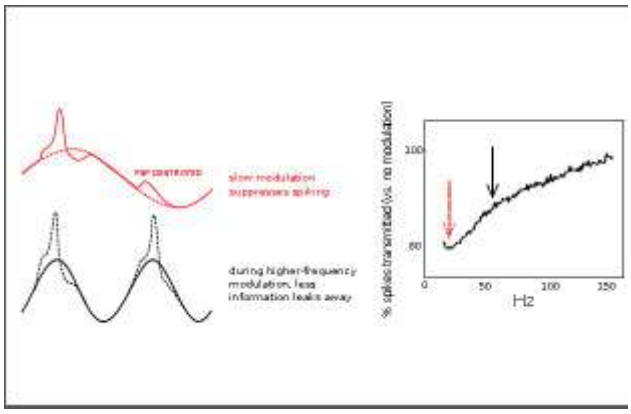
Abstract: It is widely believed that in-phase oscillatory co-modulation can generate functional enhancement between cortical regions. However, oscillatory modulation can also suppress

communication, by narrowing the available times over which neurons can fire. The extent of the suppression will depend on the modulation frequency, and its relationship to fundamental cellular timescales. For example, in the integrate-and-fire cell in the accompanying figure, 20Hz oscillatory modulation reduces the rate of transmitted spikes by 20%. During wave-troughs, post-synaptic potentials are destroyed before they can generate a downstream spikes. Yet the capacity for information transmission returns at higher frequencies (to 93% at 100Hz, and 99% at 200Hz), as more frequent wave peaks resurrect post-synaptic potentials before they dissipate. We explore this phenomenon over a series of physiological parameters, including noise level, number of neurons, average spike rate and refractory period. This kind of bandwidth reduction via low-frequency modulation may help explain the differing roles of local field potentials at different frequencies in the cortex. Whereas gamma-band (30-100Hz) oscillations have been reported to enhance functional interaction between visual areas, beta-band (15-30Hz) oscillations characteristic of parieto-frontal planning activity may suppress information transmission.



Disclosures: C.A. Stetson: None.

Poster

236. **Brain Wellness**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 236.01/I9

Topic: C.01. Brain Wellness

Title: Effects of Nelumbinis Semen on the sleep-wake cycle through the regulation of GABA and 5-HT system in rats

Authors: *H.-J. PARK[1], H. SHIM[2], Y. AHN[2], H. JEONG[2], Y.-S. CHOE[2], H. BAE[3], S. CHUNG[4], I. SHIM[2];
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Abstract:
In our previous studies, we reported the anti-stress and anti-depressant effect of Nelumbinis Semen (NS) in the rats. In the present study, we investigated the sleep facilitating effect of NS by regulating neurotransmitter systems in rats. In this study, we assessed the hypnotic effects of NS in the locomotor activity and electroencephalogram (EEG). We also assessed the receptor binding assay to the well-known molecular targets for sedative response, $GABA_A$-BZD, 5-HT_{2C}, and adenosine receptor biding test. Extract of NS produced inhibitory effect on caffeine-induced locomotor activity and theta activity. NS also significantly decreased sleep latency and sleep duration time in rats. In the binding assay, NS had high affinity to $GABA_A$-BZD and the 5-HT_{2C} receptor, but not adenosine receptor. NS has the sedative-hypnotic activity possibly by modulating $GABA_A$ and 5-HT_{2C} receptors. NS can be useful as a hypnotic, having not only sleep inducing effects, but also sleep quality-increasing effects.

Disclosures: H. Park: None. **H. Shim:** None. **Y. Ahn:** None. **H. Jeong:** None. **Y. Choe:** None. **H. Bae:** None. **S. Chung:** None. **I. Shim:** None.

Poster

236. **Brain Wellness**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 236.02/I10

Topic: C.01. Brain Wellness

Title: Severe malnourishment modifies the circadian cycle of DRD1 and DRD2 genes in the dorsal striatum and ventral tegmental area of the rat

Authors: C. LÓPEZ-OVIEDO[1], A. BOYZO-MONTES DE OCA[2], H. N. MORENO-SANDOVAL[1], J. AYALA-DÁVILA[2], L. C. SÁNCHEZ-PEÑA[3], B. A. LEÓN-CHÁVEZ[4], D. MARTÍNEZ-FONG[2], *J. A. GONZALEZ-BARRIOS[1];
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Abstract: Anorexia nervosa (AN) is a serious psychiatric disorder of unknown etiology. Recently, alterations in dopamine (DA) transmission have been implicated in its etiology and

development. Some studies have shown that diet restriction reduces DA levels in the hypothalamus, hippocampus and the dorsal striatum, and have linked the motor hyperactivity following diet restriction with increased DA levels in the hypothalamus. Neuroendocrine studies suggest that DA neurotransmission is increased in AN. Supporting this suggestion, recent neuroimaging studies have shown an increased density of dopaminergic receptors in the striatum. To determine whether food intake restriction in a model of AN with severe malnourishment modifies the circadian expression of DA receptors, we evaluated the changes in mRNA transcription of D1-like receptors (D1 and D5) and D2-like receptors (DRD2, DRD3 and DRD4) in the dorsal striatum and ventral tegmental area (VTA) along the day. A transcriptional study was conducted in cDNA samples obtained from male Wistar rats, maintained in constant light-dark cycles of 12-12 h and food intake restriction (10 g/day). The control group were rats feeding ad libitum. Dorsal striatum and VTA were dissected each 2 h starting at 8:00 AM and ending at 6:00 AM of the following day to cover a period of 24 h. The change in gene transcription were assessed by RT-PCR and real-time PCR using TaqMan probes. The severe malnourishment significantly increased DRD1 gene transcription in the VTA ($P < 0.001$) only. DRD2 transcription was up-regulated in the VTA from 22:00 to 04:00 h, whereas in the dorsal striatum was down-regulated ($P < 0.001$) in all times evaluated. DRD3 and DRD5 showed similar transcriptional profile to those of the controls rats. No transcriptional activity of DRD4 gene was detected . Our results support the hypothesis that the increase in DRD2 gene transcription in the dorsal striatum and VTA is responsible for the hyperactivity during food intake restriction period, and suggest that the DRD1 up-regulation in the VTA might account for some of the neuropsychological disorders observed in patients with AN.

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Poster

236. **Brain Wellness**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 236.03/I11

Topic: C.01. Brain Wellness

Support: This study was funded by Shire Pharmaceuticals, UK

Title: Effect of lisdexamfetamine in a rat model of binge-eating disorder

Authors: *S. P. VICKERS[1], D. J. HEAL[1], D. HACKETT[2], P. H. HUTSON[3];
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Abstract: Binge-eating disorder (BED) is a common psychiatric condition, affecting ~2% of the adult population. It occurs approximately equally in women and men and most often in older adults. It manifests as compulsive, excessive consumption of highly palatable foods and may or may not be associated with obesity. Binge eaters frequently experience intense feelings of guilt and anxiety after a binge session, but do not indulge in purging. lisdexamfetamine dimesylate (LDX; Vyvanse®), a novel prodrug that is metabolized to d amfetamine (d-AMF) primarily by red blood cells (Pennick, 2010, Neuropsychiat Dis Treat 6:317) is approved in the USA, Mexico and Europe for ADHD (ages 6-17). It is being clinically evaluated for managing BED in USA. This study compared the acute effects of LDX in rats trained to binge eat chocolate with its active metabolite, d AMF, and with sibutramine (SIB), which is reported to be moderately effective in clinical trials of BED (Wilfley et al, 2008, Am J Psychiat 165:51-58).
Forty-four adult, lean, female Wistar rats were housed individually on reversed-phase lighting with free access to standard diet and water. Ground milk chocolate was offered to each rat for 2 hour periods at irregular intervals to establish binge eating. LDX, d AMF and SIB were administered orally.
Irregular, limited access to chocolate for ~4 weeks produced reproducible binge eating, but bodyweights were not different from control rats maintained on a standard diet. LDX (0.1-1.5 mg/kg d AMF base) reduced chocolate bingeing by up to 86.1% at the highest dose ($p<0.001$). The intermediate dose of 0.3 mg/kg, LDX reduced chocolate consumption 40.2% ($p<0.001$) whilst having no effect on standard diet intake. LDX treatment did not decrease bodyweight compared with the control group given vehicle. d AMF (0.1 1.0 mg/kg d AMF base) decreased chocolate bingeing at 0.5 mg/kg ($p<0.01$) and 1.0 mg/kg ($p<0.001$). d AMF did not reduce standard diet consumption, but did reduce bodyweight ($p<0.01$) at the highest dose. SIB (0.3 5.0 mg/kg) reduced chocolate bingeing ($p<0.001$ at 1-5 mg/kg); however there were similar reductions of standard diet consumption for all doses ($p<0.05$-0.01). SIB also produced small bodyweight decreases ($p<0.05$-0.001 at 1-5 mg/kg).
Animals allowed irregular, limited access to chocolate developed robust, intermittent hyperphagia that mirrored BED without the associated obesity. Binge eating was markedly reduced by a single treatment with LDX or its metabolite, d AMF. Unlike SIB, both LDX and d AMF reduced chocolate binge-eating without simultaneously decreasing the consumption of normal diet. These results provide support for use of LDX in the clinical treatment of BED.

Disclosures: S.P. Vickers: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Shire Pharmaceuticals Limited, Basingstoke RG24 8EP, UK. **D.J. Heal:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and

pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Shire Pharmaceuticals Limited, Basingstoke RG24 8EP, UK. **D. Hackett:** None. **P.H. Hutson:** None.

Poster

236. **Brain Wellness**

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Program#/Poster#: 236.04/I12

Topic: C.01. Brain Wellness

Support: NIH Grant AG027342

The RCS Alzheimer's Foundation

Title: Iron content of choroid plexus may increase with age

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Abstract: Objective
Iron is the most abundant transition metal in brain and is essential for various cell functions, including ATP production and myelination. However, iron can promote damaging oxidative reactions. Brain iron increases with age and is a risk factor for age-related degenerative brain diseases such as Alzheimer's disease (AD). Choroid plexus (CP) is the main source of brain transferrin, the major iron delivery protein for neurons. CP undergoes progressive age-related senescence which may contribute to iron dysregulation, iron-mediated toxicity, and the increase in oxidative stress and inflammation that occurs with brain aging. CP dysfunction has been implicated in AD; however, CP calcifications do not seem to be involved. The role of CP in brain aging and disease is relatively unexplored so we assessed whether CP Susceptibility Weighted Imaging (SWI) signals, and therefore possibly iron levels, change in healthy individuals throughout their lifespan.
Methods
We estimated CP iron levels in a large sample of healthy men (N=96) and women (N=84) aged 18 to 89 years old (mean 51.8, sd=22.0). CP iron was assessed using SWI, a magnetic resonance imaging technique that is sensitive to paramagnetic substances including iron. Qualitative ratings of SWI hypointensity severity and counts of the number of slices in which CP hypointensities were observed were obtained by a single rater (ABA), blind to subject demographics. Brain iron

was assessed in putamen (P) using field dependent relaxation rate increase (FDRI).

Results

The prevalence of SWI hypointensities was 89%. Ratings of hypointensity significantly increased with age in the whole sample ($r=.41$, $p<.0001$) and in men ($p=.0043$) and women ($p<.0001$) separately. Ratings of zero (no evidence of susceptibility effects) were observed only in subjects under 65 years of age (chi squared for a rating of zero for individuals under vs. over 65 years was 13.03, $p=.0003$). Ratings of extent of hypointensity (number of slices with hypointensity) were also significantly correlated with age in the whole sample ($r=.48$, $p<.0001$) and in men ($p<.0001$) and women ($p<.0001$) separately. Correlation between CP slice ratings and P FDRI was $r=.27$, $p=.01$.

Conclusions

Qualitative estimates of CP SWI hypointensity are correlated with age. CP hypointensity may serve as a surrogate for increased deposition of iron, as the prevalence of hypointensities is much higher than reports of CP calcification prevalence. This interpretation is also supported by the correlation with P FDRI. The data supports the hypothesis that CP undergoes age-related changes that may contribute to the risk of developing age-related degenerative brain diseases such as AD.

Disclosures: T.A. Tishler: None. **A.B. Aronson:** None. **P.H. Lu:** None. **P. Villablanca:** None. **J.P. Finn:** None. **G. Bartzokis:** None.

Poster

236. **Brain Wellness**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 236.05/I13

Topic: C.01. Brain Wellness

Support: NIH R01 HD050735

NHMRC 486682

NHMRC 1009064

NHMRC 389875

NIH R01 EB008432

NIH R01 EB008281

NIH T32MH073526-06

Title: Obesity gene NEGR1 is associated with white matter integrity differently in young and old adults

Authors: *E. L. DENNIS[1], M. BRASKIE[1], N. WARSTADT[1], N. JAHANSHAD[1], O. KOHANNIM[1], T. NIR[1], K. MCMAHON[2], G. DE ZUBICARAY[3], G. MONTGOMERY[4], N. MARTIN[4], A. TOGA[1], M. WRIGHT[3,4], P. THOMPSON[1];
[1]Lab. of Neuroimaging, UCLA, Los Angeles, CA; [2]Ctr. for Advanced Imaging, [3]Sch. of Psychology, Univ. of Queensland, Brisbane, Australia; [4]Queensland Inst. of Med. Res., Brisbane, Australia

Abstract: Obesity is a major public health issue in the developed world. Obesity is associated with many health issues, and high body mass index (BMI) in midlife has been linked to decreased cognitive functioning in old age. Several studies report abnormalities in white matter volume or diffusivity associated with obesity. This is likely due in part to genes that affect both the brain and obesity risk. We investigated whether common variants in obesity-associated genes might also be associated with brain measures.
We scanned 409 subjects (264 females/145 males, mean age=23.8, range=20-29 years) at 4T with 105-gradient HARDI (high angular resolution diffusion imaging) and T1-weighted MRI. We began with a multi-locus approach that models the combined effect of a number of SNPs (single nucleotide polymorphisms) associated with obesity. This is a novel approach to examine the aggregate influence of genetic variants. We then performed a voxel-wise fractional anisotropy (FA) analysis on the SNP that appeared to be driving the association. We controlled for age, sex, BMI, and kinship (in our twin sample). The sample was split in half to test reproducibility of these results. Additionally, we attempted to replicate our results in an older group from the ADNI2 cohort (78 subjects, 29 females/49 males, mean age 74.3, range=55-90). In our initial multi-locus analysis, our BMI SNP panel was significantly associated with FA in the bilateral posterior corona radiata. The SNP appeared to be driving the association was in NEGR1 (rs2815752). A follow-up analysis yielded lower FA with risk allele dosage across extensive areas of white matter (see Figure 1). This association was robust in our split-sample replication. In the ADNI2 cohort, the same areas were associated with NEGR1 risk allele dosage, but in the opposite direction. Previous research has found that midlife obesity can be cognitively detrimental, while late-life obesity can be cognitively protective. Our results mirror this trajectory, and may be part of the mechanism underlying it.

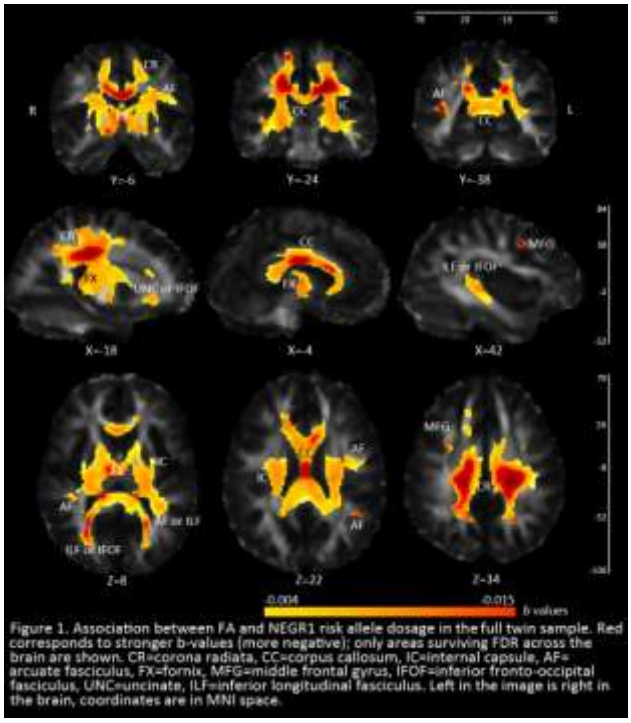

Figure 1. Association between FA and NEGR1 risk allele dosage in the full twin sample. Red corresponds to stronger b-values (more negative); only areas surviving FDR across the brain are shown. CR=corona radiata, CC=corpus callosum, IC=internal capsule, AF= arcuate fasciculus, FX=fornix, MFG=middle frontal gyrus, IFOF=inferior fronto-occipital fasciculus, UNC=uncinate, ILF=inferior longitudinal fasciculus. Left in the image is right in the brain, coordinates are in MNI space.

Disclosures: E.L. Dennis: None. **M. Braskie:** None. **N. Warstadt:** None. **N. Jahanshad:** None. **O. Kohannim:** None. **T. Nir:** None. **K. McMahon:** None. **G. de Zubicaray:** None. **G. Montgomery:** None. **N. Martin:** None. **A. Toga:** None. **M. Wright:** None. **P. Thompson:** None.

Poster

236. **Brain Wellness**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 236.06/I14

Topic: C.18. Drugs of Abuse and Addiction

Support: the Korea Healthcare Technology R&D Project, Ministry for Health and Welfare, South Korea A120157

Title: Theta-phase gamma-amplitude coupling detects EEG abnormalities in persons with internet addiction

Authors: *J. LEE[1], S.-W. CHOI[1], S. KIM[2];
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Abstract: Objective: Internet addiction disorder (IAD) is increasingly recognized as a legitimate clinical disorder and social problem that critically require treatment, yet its pathophysiological mechanism is not well understood. The aim of this study was to determine if cross-frequency phase-amplitude coupling of the electroencephalography (EEG) detects possible abnormalities in functional connectivity in IAD patients.
Methods: 19-electrode EEGs were recorded from 16 IAD patients and 35 healthy subjects. Their cross-frequency phase-amplitude coupling was estimated to compare with clinical measures including daily internet usage (DIU), internet addiction test scores (IAT), Barrett Impulsiveness scores (BIS), Beck depression inventory scores (BDI) and Beck anxiety inventory scores (BAI).
Results: The IAD group showed decreased theta-phase gamma-amplitude coupling (TGC) as well as increased delta and theta powers, compared with the healthy control group. Further, TGC negatively correlated with IU,
IAT, BIS, BDI and BAI in frontal, central, parietal and occipital regions.
Conclusions: The TGC is a valuable marker for abnormal interactions of functional brain networks in IAD patients. We suggest that such abnormal interactions among large-scale brain networks are responsible for the impulsive or maladaptive behavior that is associated with internet addiction.
Significance: The TGC could be a promising neurophysiological indicator for diagnosing the internet addiction.

Disclosures: J. Lee: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; a grant from the Korea Healthcare Technology R&D Project, Ministry for Health and Welfare, South Korea (No. A120157). **S. Choi:** None. **S. Kim:** None.

Poster

236. **Brain Wellness**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 236.07/I15

Topic: F.01. Human Cognition and Behavior

Support: Japan’s Forestry Agency Grant

Kyushu University Interdisciplinary Programs in Education and Projects in Research Development

Title: Restorative effect of wooden materials on electrophysiological responses in humans

Authors: *S. MATSUMOTO[1], T. OKAMOTO[2], A. YAMAMOTO[1], K. OHNUKI[5], T. MITSUDO[3], H. ISHIKAWA[6], R. FUKUDA[1], K. FUJITA[1], T. NAKASHIMA[7], Y. YAMADA[8], J. NAGANO[4], S. YASUNARI[9], Y. WATANABE[10], N. FUJIMOTO[1], K. SHIMIZU[1];
[1]Fac. of Agr., [2]Fac. of Med. Sci., [3]Fac. of Information Sci. and Electrical Engin., [4]Fac. of arts and science, Kyushu Univ., Fukuoka, Japan; [5]Sch. of Humanity-Oriented Sci. and Engin., Kinki Univ., Iizuka, Japan; [6]Intl. Col. of Arts and Sci., Fukuoka Women's Univ., Fukuoka, Japan; [7]Neurotechnology Res. Group, Natl. Inst. of Advanced Industrial Sci. and Technol., Tsukuba, Japan; [8]Res. Inst. for Time Studies, Yamaguchi Univ., Yamaguchi, Japan; [9]Yasunari Corp., Shimonoseki, Japan; [10]Trywood Company, Limited, Hita, Japan

Abstract: Wooden houses are known to exert some psychological effects due to their odor, humidity conditioning, and the visual elements of interior designs (Nyrud & Bringslimark, 2010, Rice *et al*., 2006, Tsunetsugu *et al*., 2007). In particular, naturally processed woods (NPW, free from chemical processing during lumber sawing) are more restorative than chemically processed woods (CPW, bonded or coated woods) (Jonsson *et al*., 2008). However, the physiological effects of wooden materials at the level of processing are still unclear. In order to clarify the difference in physiological effects between NPW and CPW, we built two experimental huts. One was made of Japanese cedar, *Tsuesugi* (NPW), and the other was made of CPW. The electrophysiological responses of subjects were examined by comparing electroencephalograms (EEG) measured in each hut, both while the subjects were at rest and during a three-stimulus visual oddball task. Ten male students (20–22 years old) participated in this experiment, and each subject came to the two huts on separate days. The measurement was conducted as follows: The resting EEG was recorded continuously for 10 minutes both before and after the task, when subjects lay sprawled out on the floor with their eyes closed. All subjects also completed three 10-minute blocks of the task while seated comfortably in a chair in front of a cathode ray tube display. Significant effects of chemical processing were observed on delta (0.5–3.5 Hz), theta (4–7 Hz), and gamma (30–55 Hz) frequencies as reflected by changes in the resting EEG. The mean amplitudes of the delta-band and theta-band activities did not change between the pre and post periods in the NPW hut, whereas they did increase from pre to post in the CPW hut. The amplitudes of gamma-band activity also did not change in the NPW hut, whereas they decreased in the CPW hut. These results can be interpreted as follows: In the NPW hut, the subjects recovered from the fatigue after the sustained visual oddball task. By contrast, in the CPW hut, the subjects experienced continuous fatigue after the task. From these results, we suggest that NPW may have a restorative effect on brain fatigue.
First three authors equally contributed to this work. We thank Prof. H. Shiratsuchi (Pref. Univ. Kumamoto) for volatile analysis.

Disclosures: S. Matsumoto: None. **T. Okamoto:** None. **A. Yamamoto:** None. **K. Ohnuki:** None. **T. Mitsudo:** None. **H. Ishikawa:** None. **R. Fukuda:** None. **K. Fujita:** None. **T. Nakashima:** None. **Y. Yamada:** None. **J. Nagano:** None. **S. Yasunari:** None. **Y. Watanabe:** None. **N. Fujimoto:** None. **K. Shimizu:** None.

Poster

236. **Brain Wellness**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 236.08/I16

Topic: C.01. Brain Wellness

Support: VA Research Service

Title: Incidence and impact of depression in blast versus non-blast traumatic brain injury in a veteran population

Authors: *G. V. WINDMILLER[1], K. PANIZZON[1], S. JOO[2], R. WALLIS[1];
[1]VA Greater Los Angeles Healthcare Syst., Los Angeles, CA; [2]VA Greater Los Angeles Healthcare Sysytem, Los Angeles, CA

Abstract: OBJECTIVE: To evaluate the incidence and impact of depression in subjects with traumatic brain injury (TBI) from blast versus non-blast mechanisms in a veteran population. BACKGROUND: Many veterans from the Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) conflicts with TBI have had blast exposure. TBI is associated with a wide array of residual effects, including post-concussive headaches, cognitive dysfunction, and depression. The effects of depression in TBI are particularly disabling. We evaluated the incidence of depression and suicidal ideation in subjects with blast versus non-blast TBI. METHODS: We performed a pilot retrospective chart review of subjects with TBI of greater than one year duration. We confirmed the history of blast or non-blast exposure TBI for each subject. We also examined the incidence of depression and suicidal ideation in these subjects. RESULTS: We reviewed the charts of 186 subjects with TBI. We found that blast exposure occurred in 108 (58%) subjects with TBI, and non-blast TBI in 78 (42%) subjects. Depression was diagnosed in a mean of 81% ± 1 of subjects with blast exposure, while depression was seen a mean of 72% ± 1 of subjects with non-blast TBI. The incidence of suicidal ideation occurred with 24% ± 1 in subjects with non-blast TBI and 22% ± 1 subjects with blast TBI. CONCLUSION: In this study, blast exposure was found to have occurred frequently in this veteran population. Also, depression occurred with a greater incidence in subjects with blast

exposure during TBI. These initial data suggest that blast injury may have a major impact on the psychological well-being of subjects exposed to this mechanism of TBI.

Disclosures: G.V. Windmiller: None. **K. Panizzon:** None. **R. Wallis:** None. **S. Joo:** None.

Poster

236. **Brain Wellness**

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Program#/Poster#: 236.09/I17

Topic: C.01. Brain Wellness

Support: NSFC 31271288

NSFC 81171238

Research Foundation for 100 Academic and Discipline Talented Leaders of Chongqing, P. R. China

Title: Exercise has positive effects on the capillaries in the white matter of aged brain

Authors: *Y. TANG, C. HUANG, X. QIU, L. CHEN, L. ZHANG, F. CHAO;
Dept. of Histology and Embryology, Chongqing Med. Univ., Chongqing, China

Abstract: Our previous studies found that during aging process, the white matter was significantly shrunk and the myelinated fibers in the white matter were markedly lost. Our previous studies also found that running exercise had positive effects on the white matter and the myelinated fibers in the white matter of aged brain. What are the reasons for the effects of running exercise on the white matter of aged brain? The present study is the first study to investigate the age-related changes of the capillaries in the white matter and the effects of running exercise on the age-related changes of the capillaries in the white matter of Sprague Dawley rats using the immunohistochemistry technique and the unbiased stereological techniques. For the investigation of the age-related changes of the capillaries in the white matter, young rats and aged rats were used. For the investigation of the effects of running exercise on the capillaries in the white matter of aged brain, 14-month-old male and female rats were randomly divided into running group and control group. Control group rats were reared in standard condition without running. Running group rats run 4 months and 14 months. The spatial learning capacity of running rats and control rats were assessed with Morris Water Maze. The white matter volume, the total volume, total length and total surface area of the capillaries in white matter were quantitatively investigated with the immunohistochemistry and the unbiased

stereological methods. The total length, total volume and total surface area of the capillaries in the white matter of aged rats were significantly lower than those of young rats. Running exercise improved the spatial learning and memory ability of aged rats. After 4 month running exercise, the total length of the capillaries in the white matter of male and female exercised rats was significantly higher than that of male and female non-exercised rats. After 14 month running exercise, the total length, total volume and total surface area of the capillaries in the white matter of male and female exercised rats were significantly higher than those of male and female non-exercised rats. The age-related changes of the capillaries in the white mater may have important implications for age-related white matter atrophy and age-related cognitive impairments. The present results indicated that running exercise had positive effects on the capillaries in the white matter of aged brain, which might be one of the structural bases for the running exercise-induced improvement of the spatial learning ability. These results together demonstrated that running exercise could benefit aged brain.

Disclosures: Y. Tang: None. **C. Huang:** None. **X. Qiu:** None. **L. Chen:** None. **L. Zhang:** None. **F. Chao:** None.

Poster

236. **Brain Wellness**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 236.10/I18

Topic: C.18. Drugs of Abuse and Addiction

Support: FAPESP

CNPq

CAPES

AFIP

Cristália

Title: The daily fix: Habitual caffeine doses improve performance in attention and executive functions irrespective of food intake

Authors: *J. L. MARIANO, J. C. F. GALDURÓZ, S. POMPÉIA;
Psicobiologia, Univ. Federal De Sao Paulo, Sao Paulo, Brazil

Abstract: Despite there being some consensus that attention/executive functions and mood/somatic symptoms are positively affected by caffeine in habitual consumers, the nutritional status under which this occurs and the doses that exert these affects are unclear. One possible reason is that studies that have investigated these effects usually include both fasting and caffeine deprivation, factors known to alter cognitive functioning and mood/somatic symptoms. As consumers vary in their habitual caffeine intake and caffeine can modulate blood glucose, it is plausible that differences between caffeine and control conditions may be due to variable reversal of adverse withdrawal effects and fasting. To address this issue, we evaluated the effects of the habitual breakfast dose of caffeine reported by each subject (baseline dose, as the experimental procedures began at 8 am) and a standardized meal (cereal bars), alone or in combination, on: a) mood/somatic symptoms through validated questionnaire (Positive and Negative Mood States, Visual Analogue Mood Scales and Bodily Symptoms Visual Analogue Scales); and b) cognitive performance [sustained and simple attention (Psychomotor Vigilance Test), and 6 executive domains - inhibition and updating (Random Number Generation task), shifting cost (Plus-Minus task), planning (Zoo Map Task), access to long term memory (phonemic and semantic fluency) and dual-task coordination-]. This was a double-blind, placebo-controlled, independent-groups design study including 60 young, healthy male participants who were randomly assigned to one of four treatments: placebo/fasting, caffeine/fasting, placebo/meal, caffeine/meal. Testing was carried out at theoretical peak-plasma concentration of caffeine and blood glucose. Regardless of fasting or meal intake, caffeine significantly improved sustained attention, simple reaction time and decreased number of lapses in an attentional task (PVT). It also improved executive updating, phonemic fluency (in the first 15 s) and subjective somatic symptoms (fatigue, weakness, dry mouth, sweating, and subjective somnolence after the 10-minutes PVT task). The meal intake did not affect performance or mood/somatic symptoms. These findings show that caffeine-induced improvement in various aspects of attention and some executive domains in habitual users is not dependent on food intake and suggest that it may be due to reversal of abstinence or to the fact that users can determine the optimum caffeine dose that leads to enhanced performance.

Disclosures: J.L. Mariano: None. **J.C.F. Galduróz:** None. **S. Pompéia:** None.

Poster

237. **Abeta Toxicity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 237.01/J1

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: CIHR Grant

Dean's PhD Scholarship for Graduate Research

Title: The role of a nuclear localized 82 kDa choline acetyltransferase in cell stress and the epigenome

Authors: *W. WINICK-NG, R. J. RYLETT;
Phys/Pharm, Western Univ., London, ON, Canada

Abstract: The current studies were designed to explore the functional role of an 82 kDa isoform of choline acetyltransferase (M-ChAT) that is localized in the nucleus of human cholinergic neurons. Retinoic acid differentiated SH-SY5Y neuroblastoma cells were either stably-transfected with M-ChAT or transiently-transfected with a GFP-MChAT fusion protein, then treated with oligomers of amyloid β (Aβ) 1-42 for 4h. Live GFP-MChAT transfected cells were stained with Hoechst stain then imaged by confocal microscopy, or stably-transfected cells were treated with Hoechst stain either prior to or following formalin fixation. MChAT is localized predominantly to the nucleus and has a punctate-like appearance. Following 4h exposure of SH-SY5Y cells expressing M-ChAT to oligomeric Aβ[1-42], aggregations of ChAT protein appear in the nucleus. The distribution of these aggregates was altered depending on how the cells were stained with Hoechst. Applying Hoechst following fixation produces aggregations of M-ChAT that are surrounded by the dye, while applying Hoechst to live cells produces aggregations of dye that are surrounded by accumulations of M-ChAT. Z-stack 3D-renderings of Aβ treated cells show the same pattern of expression. Treatment with the histone deacetylase inhibitor trichostatin A with or without 4h Aβ[1-42] also showed the same Hoechst-dependent M-ChAT distribution change. Treatment for 24h with the histone acetyltransferase inhibitor curcumin in the presence of Aβ following 4h with Aβ alone attenuated the aggregations of M-ChAT. Primary cortical neurons cultured from embryonic mice that are doubly transgenic for mutant human APP and Presenilin were transduced with adenovirus encoding the M-ChAT protein. These neurons showed similar nuclear aggregations of M-ChAT following 10 days in culture. Taken together, these data suggest that M-ChAT protein aggregates in the nucleus following Aβ-induced cell stress, and these aggregates are displaced by Hoechst stain. Further, ChAT accumulation may be a result of increases in histone acetylation, but additional studies are required to confirm this and to determine whether these accumulations are a result of DNA binding or other mechanisms. These studies were funded by a CIHR grant to RJR and a Dean's PhD Scholarship for Graduate Research to WW.

Disclosures: W. Winick-Ng: None. **R.J. Rylett:** None.

Poster

237. **Abeta Toxicity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 237.02/J2

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: KAKENHI 21700415

High-Tech research center (TWIns)

Consolidated Research Institute of Advanced Science and Medical Care (ASMeW)

Global COE 'Practical Chemical Wisdom' projects

Leading Graduate Program in Science and Engineering

Title: Arctic mutant Aβ modifies CHRNA7's functions through specific binding

Authors: *Y. JU[1], T. ASAHI[2,3], N. SAWAMURA[2,3];
[1]Sch. of Advanced Sci. & Engineering, Waseda Univ., Tokyo, Japan; [2]Advanced Sci. & Engin., Tokyo, Japan; [3]ASMeW, Tokyo, Japan

Abstract: Point mutations within the Amyloid β-protein (Aβ) sequence that are associated with hereditary disease similar or identical to AD are clustered around the central hydrophobic core of Aβ. The nicotinic acetylcholine receptors, key players in neuronal communication, convert neurotransmitter binding into membrane electrical depolarization. CHRNA7 is a type of the neuronal nicotinic receptors and thought to have association with Alzheimer's disease, because Aβ 42 is reported binding to CHRNA7 protein with high affinity. Therefore, we came up with the hypothesis about mutant Aβ modifies CHRNA7's function which could relate to the familial Alzheimer's disease.

We utilized immunoprecipitation to detect the in vitro binding ability of several mutant Aβ with CHNRA7 and found out that Arctic mutant Aβ specifically bound to CHRNA7. We were also able to observe the aggregation form of Arctic mutant Aβ with addition of CHRNA7 through transmission electron microscopy (TEM). CHRNA7 is the cholinergic receptor controlling cellular calcium ion homeostasis and ERK1/2 protein is involved in CHRNA7 function. Thus, we detected whether Arctic Aβ could affect permeability to calcium ions. As a result, we found out that Arctic Aβ could inhibit the CHRNA7 function via decrease in calcium flux and inhibition of ERK1/2 activation.

Based on these results, we are confirming the hypothesis that Arctic mutant Aβ inhibit activation of CHRNA7 and related to familial Alzheimer's disease through binding and aggregation.

Disclosures: Y. Ju: None. **T. Asahi:** None. **N. Sawamura:** None.

Poster

237. **Abeta Toxicity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 237.03/J3

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: ANR RPIB, Neuroscreen, FR

Foundation plan Alzheimer, Model systems

Title: The role of caspases in axonal degeneration and synapse loss

Authors: *E. JACOTOT[1], B. DELEGLISE[2], B. LASSUS[2], V. SOUBEYRE[2], E. DUPLUS[2], J.-M. PEYRIN[2], B. BRUGG[2];
[1]CNRS UMR 7102, Inserm, Paris, France; [2]UMR 7102 UPMC, CNRS, Paris, France

Abstract: Axonal and synaptic degeneration are key events in peripheral neuropathies and CNS neurodegenerative diseases. In numerous physiological and pathological conditions, caspases, the effector proteases of apoptosis, are key executionner of neuronal cell body (soma) death. Distinct degenerative processes have been identified for axons, and apoptotic biochemical cascades can be activated locally in synapses, axons, and dendrites, suggesting a role for such local apoptotic signals in neurodegenerative disorders. Although a role for caspases in axon degeneration was initially widely discounted, their potential involvement was however revisited recently due to the genetic and biochemical demonstration that caspase-6 and caspase-3 are implicated in trophic deprivation and developmental axon pruning. To further investigate the role of caspases in key neuronal compartments (soma, axon, synapse), we have developed microfluidic (μFD) devices adapted to neuronal structures, and allowing a fine manipulation of local microenvironment. We grew primary cortical mouse neurons in μFD devices to separate soma from axonal projections in fluidically isolated microenvironments, and applied apoptotic stresses (e.g. β-amyloid peptide, staurosporine, dKCl) locally to the different cellular compartments. We observed that such stresses applied to the somato-dendritic compartment trigger axonal degeneration, and that axonal (distal) co-treatment with the broad-spectrum caspase inhibitor z-VAD-fmk prevents axonal degeneration. Our results suggest that local (axonal) caspases are activated through an anterograde initiator signal. The role of some individual caspases will be presented. We have also designed μFD devices with funnel-shaped microchannels ("axonal diodes") allowing the *in vitro* reconstruction of an orientated functional cortico-hippocampal neuronal network. Using such neuronal networks we show that somato-dendritic deposits of Aβ-peptide on cortical neurons trigger a rapid presynaptic disconnection

followed by axonal dying back pattern. The effects of caspase inhibition in the axonal/synaptic compartment are under investigation.

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Poster

237. **Abeta Toxicity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 237.04/J4

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: ANR Maalad

Fondation Neurodis

Title: Synaptic activation or amyloid beta oligomers promote tau trafficking to spines and an interaction with actin

Authors: M. FRANDEMICHE[1], S. DE SERRANO[1], T. RUSH[1], M. DOLLMEYER[1], A. ELIE[1], I. ARNAL[1], F. LANTÉ[1], *A. BUISSON[2];
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Abstract: Alzheimer’s disease (AD) is a progressive neurological disorder that is characterized by memory loss and confusion. There is good evidence indicating that the accumulation of the β-amyloid protein (Aβ), a 4-kDa polypeptide derived from the proteolytic cleavage of the Aβ precursor protein (APP), is a primary event in the pathogenesis of AD. Another cardinal feature of AD is the presence of intraneuronal neurofibrillary tangles, composed of insoluble aggregates of the stabilizing Microtubule-Associated Protein (MAP), tau. Once believed to mediate neuronal death and cognitive deficits, observations in mouse models have since shown that tangles exert negligible neurotoxicity compared to non-aggregated (Oddo et al., 2003). The inter-relationships between the two Aβ oligomers or/and soluble tau cytopathologies still need to be characterized. Here, we studied the sub-cellular repartition of tau in cultured primary cortical neurons and hippocampal slices from adult mice following exposure to specific synaptic activation, LTP and soluble Aβo.

First, we investigated the localization of tau in primary cortical neurons (DIV 14) exposed to a synaptic activation using a GABA-A receptor antagonist (bicuculline, 50 µM) in the presence of a voltage-gated K+-channel blocker (4-aminopyridine, 2.5 mM). We then performed synaptosomal fractionation to isolate "PSD-enriched" and "non-PSD-enriched" fractions. We then adapted this protocol to the mouse hippocampal slices after inducing LTP. We showed that synaptic activation and LTP promote tau translocation into the post-synaptic density compartment (2-fold increase vs. controls). This synaptic localization of tau exploits a selective interaction with filamentous actin located into the dentritic spines as revealed by confocal live imaging and immuno-precipitation with phalloidin. Similarly, using a cell free system, we demonstrated that recombinant human tau binds to actin filaments and promotes their organization into bundles. Together, these data suggest that tau participates in a re-organization of the actin cytoskeleton involved in synaptic plasticity. Strikingly, exposure to 100 nM of Aβo (for 15 min) induced a mislocalization of tau into the spines, both during resting conditions and under synaptic activation, as observed with confocal imaging and western blot analysis of fractionated samples. These observations suggest that tau mislocation may represent an early step leading to the synaptotoxicity observed in AD.

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Poster

237. **Abeta Toxicity**

Location: Halls B-H

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Program#/Poster#: 237.05/J5

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Scottish Rite Charitable Foundation Grant 11103

Western Strategic Support for CIHR Success Award

Title: Examining the relationship between the Warburg effect and amyloid-beta resistance in a transgenic Alzheimer's disease mouse model

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Abstract: Numerous studies of post-mortem brain tissue have revealed amyloid plaque deposition in elderly individuals with no symptoms of Alzheimer's disease (AD). It is believed that asymptomatic individuals with high plaque load likely had undiagnosed mild cognitive impairment or high cognitive reserve. However, we propose that some of these individuals may have developed resistance to the toxic effects of amyloid-beta (Aβ) peptide, a principle component of plaques found within the brains of AD patients. We previously demonstrated that Aβ resistant nerve cells exhibit elevated expression of pyruvate dehydrogenase kinase 1 (PDK1), an enzyme that phosphorylates and inhibits pyruvate dehydrogenase (PDH); a rate limiting enzyme of the citric acid cycle and a central regulator of mitochondrial activity. Elevated PDK1 activity can elicit the Warburg effect (also known as aerobic glycolysis); a form of metabolism frequently employed by cancer cells as an anti-apoptotic strategy. APPswe/PSEN1dE9 double transgenic AD (tg-AD) mice accumulate amyloid plaques in the hippocampus and cortex by 4-6 months of age but exhibit minimal neuronal loss and only display cognitive decline after 9 months of age. Immunoblot analysis of cortical tissue extracts from 3 month old mice, in addition to embryonic primary cortical neuronal cultures, revealed elevated PDK1 expression in tg-AD mice relative to wildtype littermate controls. However, PDK1 expression declined in cortical tissues from 12 month old tg-AD mice compared to control mice; an event that correlated with the age-dependent cognitive decline observed in tg-AD mice. To determine if elevated PDK1 expression in young tg-AD mice contributes to Aβ resistance, we treated mice with dichloroacetate (DCA), a chemical inhibitor of PDK1. Immunoblot analysis of cortical tissue from DCA treated mice revealed decreased phosphorylation of PDH, the substrate of PDK1, compared to untreated mice, indicating that DCA can cross the blood brain barrier. In order to measure aerobic glycolysis in vivo we performed 1H magnetic resonance spectroscopy (MRS) imaging of wildtype and tg-AD mice brains with a focus on measuring lactate, a major product of the Warburg effect. Initial MRS analysis revealed that CNS lactate levels decrease following administration of DCA. We are currently attempting to determine if DCA-mediated inhibition of the Warburg effect potentiates cognitive decline and neuronal loss in young tg-AD mice. Establishing a link between the Warburg effect and Aβ-resistance in vivo may provide valuable clues as to why some individuals exhibit little or no dementia when faced with high Aβ deposition in their brains.

Disclosures: R.C. Cumming: None. **R. Bartha:** None. **R.A. Harris:** None. **J.T. Newington:** None.

Poster

237. **Abeta Toxicity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 237.06/J6

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH AG-044208

NIH AG-37337

ADDF 20100501

Title: Mechanism of internalization of Abeta oligomer-receptor complexes

Authors: *K. MOZZONI, C. REHAK, C. SILKY, N. J. IZZO, R. YURKO, H. SAFFERSTEIN, G. RISHTON, S. M. CATALANO;
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Abstract: Abeta Oligomers accelerate vesicular exocytosis in neurons without affecting endocytosis rate (Lui and Schubert '97). We studied the mechanism of vesicular trafficking deficits caused by Abeta oligomers in DIV21 rat hippocampal cultures using Pitstop2 (Abcam cat# ab120687), a small molecule inhibitor of clathrin-mediated endocytosis and dynasore (Tocris cat# 2897), a peptide inhibitor of the dynamin-dependent endocytotic scission process using measurement of a vesicular cargo dye. Pitstop2 did not affect trafficking rate when dosed from 0.25uM-20uM, nor did it change Abeta-mediated trafficking abnormalities. Dynasore treatment induced the formation of enlarged vesicles in swollen neurites and cell bodies and slowed the rate of exocytosis (EC50=3.5uM, decrease of 40%). Dynasore counteracted the acceleration of Abeta-induced exocytosis, restoring trafficking rates and neurite appearance (100% of normal at 80uM**,** EC50=2.6uM). We conclude that Abeta acts on a dynamin-independent exocytosis process. Abeta oligomers exhibit saturable oligomer binding (Kd =1.4uM) to cell surface receptors causing internalization of oligomer-receptor complexes over time (Lacor et. al '04). Immunofluorescent labeling with and without detergent was used to measure total vs. surface oligomer binding (statistical comparisons pairwise Student's t-test). Abeta oligomers bound to surface receptors are gradually internalized by endocytosis, resulting in decreased binding intensity at the cell surface (1 uM total abeta concentration; 1 hour = 3.5% ±8, $p>0.05$, 2 hours = 27% ±3.3, $p<0.05$). Pitstop2 did not significantly affect internalization of receptor-bound oligomers (1 hour = 7% ± 3, $p>0.05$, 2 hours = 12% ±1.8, $p>0.05$). Dynasore significantly increased the amount of internalized oligomers (1 hour = 27% ±1.3, $p<0.05$) but this effect was transient (2 hours = 27% ± 1.5, $p>0.05$). We conclude receptor-bound Abeta oligomer is internalized by a dynamin-dependent process. Pitstop did not significantly affect the total amount of receptor bound oligomers (1 hour= 3%±5.5, $p>0.05$, 2 hours=8% ± 3.5, $p>0.05$). Dynasore significantly decreased the total amount of receptor bound oligomers at 2 hours (17% ± 1, $p<0.05$) but did not significantly decrease the total amount in 1 hour (2% ± 1.3, $p>0.05$). We

conclude that receptor-bound Abeta oligomer binding is decreased by dynasore due to depletion of receptors that have not been recycled back to the surface of the cell.

Disclosures: K. Mozzoni: A. Employment/Salary (full or part-time):; Full-Time Employee of Cognition Therapeutics. **C. Rehak:** A. Employment/Salary (full or part-time):; Part-time employee of Cognition Therapeutics. **C. Silky:** A. Employment/Salary (full or part-time):; Full-Time Employee of Cognition Therapeutics. **N.J. Izzo:** A. Employment/Salary (full or part-time):; Full-Time Employee of Cognition Therapeutics. **R. Yurko:** A. Employment/Salary (full or part-time):; Part-Time Employee of Cognition Therapeutics. **H. Safferstein:** A. Employment/Salary (full or part-time):; Full-Time Employee at Cognition Therapeutics. **G. Rishton:** A. Employment/Salary (full or part-time):; Full-Time Employee of Cognition Therapeutics. **S.M. Catalano:** A. Employment/Salary (full or part-time):; Full-Time Employee of Cognition Therapeutics.

Poster

237. **Abeta Toxicity**

Location: Halls B-H

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Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH AG044208

ADDF 20100501

NIH AG-37337

Title: A pharmacological model for treating Abeta synaptotoxicity at a receptor target

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Abstract: Soluble Abeta oligomers are implicated in synaptic loss and memory deficits seen in Alzheimer’s disease, but the molecular target mediating the synaptotoxic effects of these oligomers has not been identified. Alterations in intracellular trafficking are key mechanism by which Abeta oligomers affect synapse plasticity (Ehler 2006). We synthesized novel, blood brain barrier penetrable small molecules that are selective, high affinity ligands for a receptor in the brain which has not been previously implicated in Alzheimer’s pathology. CT109 and CT093 compete with Abeta oligomers for binding to neurons (72% and 68% reduction in binding,

respectively), reverse the effects of Abeta oligomers on vesicular trafficking in neurons (EC50 = 2.5 uM and 4.9 uM, respectively) and reverse deficits in memory and learning in huAPP-Swe/Ldn mice. Immunofluorescent labeling of DIV21 rat hippocampal neurons demonstrates punctate localization of the receptor in DIV21 rat neuronal synapses in close juxtaposition with presynaptic synaptophysin-1 labeling. Previously characterized ligands for the receptor were used in a vesicular trafficking assay and were found to express distinct functional phenotypes. CT1357 reversed Abeta-induced effects on trafficking (EC50 = 6.4 uM) with a toxic effect above 10 uM as indicated by an inhibition of cellular metabolism (MTT, Alamar Blue) and decreases in nuclear size. CT1357 also caused cleavage of Caspase-3 and activation of Caspase-3 enzymatic activity with a maximum effect at 40 uM (fold increase = 1.95 +/- 0.27, $p< 0.001$). CT1359 reversed Abeta effects on trafficking (EC50 = 2.5 uM) but without a cytotoxic effect on the neurons and without activation of Caspase-3. CT109 and CT0093 did not induce neuronal toxicity or activation of Caspase-3 and block the activation of Caspase-3 by 84% +/- 14% and 100% +/-12%, respectively ($p<0.001$). We conclude that CT109 and CT0093 bind to a specific receptor at post-synaptic sites where they compete for binding with Abeta oligomers, blocking the synaptotoxic effects of the Abeta oligomers. These compounds are antagonists of the receptor and do not induce activation of Caspase-3 and apoptosis in neurons as do agonists of the receptor. The ability to functionally block the synaptotoxic effects of Abeta oligomers without inducing apoptosis gives CT109 and CT0093 a desired therapeutic profile of a disease modifying treatment for Alzheimer’s disease.

Disclosures: **N.J. Izzo:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics. **K. Mozonni:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics. **C. Silky:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics. **C. Rehak:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics. **R. Yurko:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. **R. Mach:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutics. **J. Xu:** None. **C. Zeng:** None. **G. Rishton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics. **H. Safferstein:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics. **S.M. Catalano:** A. Employment/Salary (full or part-time):; Cognition Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of

intellectual property rights/patent holder, excluding diversified mutual funds); Cognition Therapeutics.

Poster

237. **Abeta Toxicity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 237.08/J8

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Spreading of amyloidosis to unaffected areas of the hippocampus studied *In vivo* by nervous tissue transplantation

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Abstract: Alzheimer's disease is the most common form of dementia, presenting with a progressive cognitive impairment. It is characterized by the presence of two principal hallmarks: amyloid plaques and neurofibrillary tangles. There is growing evidence that aggregated amyloid peptides and phosphorylated Tau proteins can spread from affected to unaffected neighboring areas of the brain. In this study we tried to assess this hypothesis by using a new in vivo methodological approach. To determine whether the spread of amyloid-beta peptide (Aβ) to healthy tissue causes different cell damage compared with endogenous production, we studied the survival of solid embryonic hippocampal grafts, derived from E15 GFP+ wild-type embryos, within a period of two months after transplantation in the hippocampi of adult wild type or APP/PS1 transgenic mice, model of Alzheimer's disease. Tissues grafted in APP/PS1 hippocampi displayed a 40% decrease in the number of neurites extending into the host ($P<0.05$) and a 27% lower dendritic spine density ($P<0.05$) compared to those grafted in wild-type recipients. The reduction was more severe for stubby-shaped spines. Moreover, some amyloid plaques were detected within the tissue transplanted in APP/PS1 mice along with dystrophic neurites and activated glial cells that surrounded the amyloid deposits. Our results show that amyloid plaque deposition can propagate very quickly to previously healthy tissue, thereby inducing neurite abnormalities and gliosis, in line with the current hypothesis of a prion-like propagation of brain amyloidosis.

Disclosures: M. Sadallah: None. **F. Tempia:** None.

Poster

237. **Abeta Toxicity**

Location: Halls B-H

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Program#/Poster#: 237.09/J9

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Exosomes can bind to and neutralize the activity of plasticity-disrupting Aβ assemblies *In vivo*

Authors: Y. SON, J. JUNG, T.-Y. JEONG, J. LEE, S.-J. KIM, S. LEE, S. LEE, H.-J. JO, *J.-H. KIM;
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Abstract: Accumulated studies have indicated that oligomeric forms of amyloid beta (Aβ) interfere with long-term potentiation (LTP), a cellular correlate of learning and memory, when they are administrated to cultured neurons, acute brain slices or hippocampus regions of live animal. However, the levels of LTP inhibition markedly vary depending upon the concentration of soluble Aβs used or their status. It was previously suggested that there are several factors that can regulate Aβs in the interstitial fluid (ISF), for example, transporting proteins through blood-brain barrier, Aβ-degrading enzymes, etc. In this study, we found that the exosomes, a small secreted lipid vesicles that contain lots of membrane proteins including insulin-degrading enzyme, cellular prion proteins can ameliorate the synaptic impairment caused by oligomeric form of Aβ (ADDLs) through in vivo LTP. We also provide in vivo evidence that N2a cell-derived or human CSF-derived exosomes can neutralize the synaptic-plasticity-disrupting activity of AD brain-derived Aβ and that this effect involves sequestration of synaptotoxic Aβ assemblies by exosomal surface proteins such as PrPC.

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Poster

237. **Abeta Toxicity**

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Topic: C.03. Alzheimer's Disease and Other Dementias

Support: FONDECYT 11090091

FONDECYT 1130747

Title: P2XR activation contribute to acute Aβ excitotoxicity through an ATP-dependent mechanism

Authors: *F. SÁEZ-ORELLANA, A. DIBARRART, P. A. GODOY, C. A. RIVERA-VERA, J. GUZMAN, L. G. AGUAYO, J. FUENTEALBA;
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Abstract: Alzheimer´s Disease (AD) is the most common form of dementia in the elderly. To date, the main recognized toxic agents of the disease are the oligomeric forms of the amyloid beta (Aβ) peptide. One the proposed toxic actions of Aβ is the ability to bind to cell membranes and form non-selective pores or channel-like structures. Recent studies suggest that these pores have an inner diameter about 1.0 - 2.0 nm; large enough to allow the passage of molecules such as dyes and, more interestingly, cellular metabolites like ATP. The presence of pre-synaptic purinergic receptors that can modulate the release of neurotransmitters provide a unique feedback mechanism to promote the synaptotoxicity of Aβ. The aim of this work was to study the modulation induced by ATP on Aβ toxicity through P2X receptor activity.
Aβ induced a 2-fold increase in $[ATP]_e$ with respect to basal levels. This increase could promote the activation of P2X receptors and induce changes in $[Ca^{2+}]_I$ that can be partially blocked by PPADS (10 µM) and Apyrase (3 U/ml). We recorded miniature excitatory (mEPSC) and inhibitory (mIPSC) spontaneous post-synaptic currents pharmacologically isolated (mIPSC: TTX 25 nm + CNQX 5 µM + DAP5 50 µM;
mEPSC: TTX 25 nm + Bicuculline 5 µM) and quantified the frequency of events. The effect of Aβ on the mIPSC frequency did not show a significant decrease (Control: 100 ±18%; Aβ: 70 ±11%); whereas the mEPSC were increased 2.5-fold (Control: 100 ±15%; Aβ: 248 ±32%). These data correlate with previous results from our group that demonstrate a more elevated affinity of Aβ for excitatory versus inhibitory neurons. The effect of Aβ on the frequency of mEPSC can be prevented by the non-specific P2X blocker PPADS (10 µM) (Aβ: 248 ±32%; Aβ + PPADS: 111 ±18%) and also by Apyrase (3 U/ml) (Aβ: 248 ±32%; Aβ + Apy + DPCPX: 131 ±13%). Our results demonstrate that Aβ differentially affects glutamatergic neurons and this effect could be related to the activation of P2X receptors by a leak of ATP. The direct effects of Apyrase on Aβ oligomers and direct interaction between Aβ and P2X receptors was discarded using Thioflavin T assay and immunocytochemistry. These results can be useful to develop a new pharmacological target and drug discovery model to search for new therapies in the treatment of AD, and also for other pathologies related to ATP dyshomeostasis.

Disclosures: F. Sáez-Orellana: None. **A. Dibarrart:** None. **P.A. Godoy:** None. **C.A. Rivera-Vera:** None. **J. Guzman:** None. **L.G. Aguayo:** None. **J. Fuentealba:** None.

Poster

237. **Abeta Toxicity**

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Program#/Poster#: 237.11/J11

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Lipocalin 2 is produced in response to amyloid beta

Authors: S. D. MESQUITA, A. C. FERREIRA, A. M. FALCAO, J. C. SOUSA, T. G. OLIVEIRA, M. CORREIA-NEVES, N. SOUSA, F. MARQUES, *J. A. PALHA;
Life and Hlth. Sci. Res. Inst. (ICVS), Sch. of Hlth. Sciences, U, Braga, Portugal

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder associated with an increased brain production, accumulation and aggregation of amyloid beta (Aβ) peptides. These processes are influenced by different modulators. Iron is one of these modulators, which is particularly relevant given its ability to regulate the expression of amyloid precursor protein and to drive Aβ aggregation into toxic oligomers. Herein we describe that lipocalin 2 (LCN2), a mammalian acute-phase protein involved in iron homeostasis, is highly produced in vitro by choroid plexus (CP) epithelial cells and cortical astrocytes, in response to Aβ1-42. These findings point to a role of LCN2 on Aβ-mediated toxicity.

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Poster

237. **Abeta Toxicity**

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Topic: C.03. Alzheimer's Disease and Other Dementias

Support: FONDECYT 1100502

Title: APP and Prion interaction with Aβ

Authors: *C. M. PETERS[1], M. ESPINOZA[1], C. OPAZO[2], L. G. AGUAYO[1];
[1]Dept. de Fisiologia, Univ. De Concepcion, Concepcion, Chile; [2]Oxidation Biol. Lab., Univ. of Melbourne, Melbourne, Australia

Abstract: Alzheimer´s disease (AD) is a progressive neurodegenerative disease that affects the human brain and causes cognitive and behavioral disorders. A major characteristic of AD is the presence of β-amyloid peptide (Aβ) oligomers in the brain. We have previously shown that Aβ oligomers (Aβo) associate with the neuronal membrane and induce the formation of perforations, causing an influx of calcium ions and increasing the release of synaptic vesicles that leads to a delayed synaptic failure produced by vesicle depletion.
Recent studies suggested that Aβ interacted with several proteins of the plasma membrane, such as the amyloid precursor protein (APP) and cellular Prion (PrPc). Therefore, these proteins could be participating in some of the toxic effects of Aβ in the brain. With the aim of determining if the levels of these two proteins affect the association and the toxic effects of Aβ on hippocampal neurons, we performed a series of experiments enhancing and reducing the membrane levels of APP and PrPc. Our results show that Aβ readily associated to the plasma membrane of HEK cells and hippocampal neurons after 1 h of incubation. The use of phospholipase C (PLC) decreased the levels of membrane PrPc in hippocampal neurons and also reduced Aβo association to these neurons (Aβo 544.5 ± 6.5, Aβo plus PLC 466.5 ± 13.5 RU). On the other hand, overexpression of APP in HEK cells increased association of Aβ fibrils (Aβf) (Aβf 3.388 ± 1.249, APP overexpressed plus Aβf 16.74 ± 5.184 RU), but not the association of Aβo to the membrane (Aβo 3.180 ± 1.349, APP overexpressed plus Aβo 1.427 ± 0.608). The latter suggests that the state of Aβ aggregation is important for its interaction with other membrane proteins. Finally, we performed functional experiments using patch clamp recordings to directly evaluate the capacity of Aβ to disrupt the membrane by reducing the levels of these proteins. The results support the idea that the interaction of Aβ with APP and PrPc is critical for peptide association and membrane disruption.

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Poster

237. **Abeta Toxicity**

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Topic: C.03. Alzheimer's Disease and Other Dementias

Support: BMBF 01EV0710

BMBF 01ER0803

Title: Staged spatial correspondence between patterns of amyloid-β plaque deposition and intrinsic functional connectivity in prodromal Alzheimer's disease

Authors: *C. SORG[1], J. GÖTTLER[1], T. GRIMMER[1], M. MÜHLAU[1], A. KURZ[1], H. FÖRSTL[1], C. ZIMMER[1], A. WOHLSCHLÄGER[1], V. RIEDL[1], N. MYERS[2];
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Abstract: Accounting for widespread amyloid-ß-pathology (Aß) beyond the default mode network (DMN), we extend DMN-centered models of Aß-propagation in Alzheimer's disease (AD) by proposing Aß-pathology to disrupt functional connectivity of intrinsic brain activity (iFC) in associative hubs and then to spread out along an iFC gradient across and within intrinsic networks. This model implies that the spatial correspondence between patterns of Aβ-pathology and iFC is distinctive across and within networks in an essentially staged pattern.
Pittsburgh-Compound-B (PiB)-PET and resting-state functional MRI was used to determine regional Aβ-plaque deposition and iFC in 40 asymptomatic and symptomatic (mild cognitive impairment MCI) elderly with and without PiB-uptake. Spatial iFC-pattern of intrinsic networks were derived from fMRI data via independent component analysis. We defined global and local spatial correlation (SpC) between Aβ-plaque pattern and iFC pattern and analyzed whether (i) global-SpC is the more positive the more Aβ-plaques in the network, and (ii) local-SpC is negative in hubs affected by Aβ-plaques.
In patients, we found a positive spatial correlation between global Aβ-plaque- and iFC-patterns in several fronto-parietal networks, with strongest correlations in the DMN, followed by lateral attention-networks (ATN). Conversely, in hubs of ATNs and the DMN, local correlations between Aβ and iFC were negative and progressively reduced. Correspondingly, Aβ-plaque-deposition was higher in the DMN than ATNs or other networks.
These results provide evidence for a staged pattern of spreading Aβ-pathology along intrinsic connectivity across and within intrinsic networks in AD. Data suggest a graded network degeneration model beyond the DMN in Alzheimer's disease.

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Poster

237. **Abeta Toxicity**

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Topic: C.03. Alzheimer's Disease and Other Dementias

Support: SURE Grant to CC, MP, & SS

SFR Grant # 535 to DBG

Grenolds Memorial Fund to DBG

President's Fund for Excellence to DBG

Title: Beta amyloid, transmitter release and free radicals in cultured avian retinal cells

Authors: S. J. WALAS[1], B. STIEFEL[1], C. COUGHLIN[1], M. PAZ[1], S. SAJJAD[1], *D. GRAY[2]; [1]Biol., Simmons Col., Boston, MA; [2]Simmons Col., BOSTON, MA

Abstract: Beta-amyloid (Aβ) peptides have long been associated with senile neuritic plaques in the brains of Alzheimer's Disease (AD) patients. Previously published data using 14 day embryonic neuronal terminals in avian choroids and retinas demonstrate that Aβ peptide oligomers can significantly inhibit potassium-evoked acetylcholine (ACh) release. This modulation pathway requires nitric oxide (NO), cGMP, and activation of PKG as well as the generation of free radicals as evidenced by sensitivity to superoxide dismutase (SOD). Aβ is associated with retinas in animal models of glaucoma. To study the connection between neuromodulation and cell death, an in vitro model was developed using cultured retinal cells from the avian embryo. Retinas from 14 day chick embryos were dissociated and plated on 24 well plates coated with poly-ornithine and incubated with DMEM (Dulbecco's Modified Eagle Medium) and chick embryo extract. Survival, basal and evoked ACh release were measured and compared to values after exposure to aged soluble oligomers of Aβ with and without various pharmacological treatments. Cell survival was optimal over 4 days in culture. Potassium-evoked, calcium-dependent ACh release was significantly inhibited with overnight exposure to Aβ oligomers without obvious decreases in cell survival. Studies quantitating neuronal death of cultured avian retinal cells in conditions described above are underway. The presynaptic inhibition by Aβ was sensitive to inhibitors of nitric oxide synthase and was mimicked by nitric oxide donors. These results are similar to those in all other cholinergic neuron preparations studied by this lab, suggesting that Aβ may serve as a universal cholinergic modulator. Addition of superoxide dismutase also decreased the Aβ effect implying that free radical formation may be required for this type of synaptic modulation. One of the most common and toxic free radicals formed by combination of nitric oxide and mitochondrial superoxides is peroxynitrite. However, a specific inhibitor of peroxynitrite, 5-aminosalicylate, had no effect on transmitter release or its inhibition by Aβ. These results suggest that it may be possible to separate the neuromodulatory actions of Aβ from its associated pathology.

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Poster

237. **Abeta Toxicity**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 237.15/J15

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: The Aβ oligomer count in CSF is a biomarker for Alzheimer's disease

Authors: *D. WILLBOLD[1,2], L. WANG-DIETRICH[3], O. BANNACH[2], E. BIRKMANN[3], S. A. FUNKE[3];
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Abstract: Alzheimer's disease (AD) is a fatal neurodegenerative and progressive disorder. Currently, no reliable biomarkers for pre-symptomatic diagnosis or therapy monitoring are available. Recent studies indicate that especially soluble amyloid beta peptide (Aβ) oligomers are the major toxic species during development and progression of AD. Therefore, we suggest that the number of Aβ oligomers in body fluids can be used as the most relevant and direct biomarker for AD.
Our new surface-based fluorescence intensity distribution analysis (sFIDA) assay for quantification of Aβ oligomers with single particle sensitivity, is able to count Aβ oligomers in human cerebrospinal fluid (CSF). We challenged the assay with CSF samples from 14 AD patients and 12 age-matched control subjects. The Aβ oligomer count revealed a surprisingly clear distinction between both groups. All samples of the control group showed homogenously low numbers of Aβ oligomers, while the samples of the AD group exhibited significantly higher levels of Aβ oligomers. The Aβ oligomer levels clearly correlated with the patients' mini-mental state examination (MMSE) scores.
Our results support the idea that Aβ oligomers play a crucial role in AD pathology and in turn can be used as a diagnostic biomarker. The sFIDA assay is able to reliably quantify the Aβ oligomers in human CSF. In addition, the correlation between MMSE scores and Aβ oligomer counts suggests that the quantity of Aβ oligomers in CSF correlates with the severity of the disease. This will allow to evaluate and to monitor anti-Aβ-targeted therapies based on the Aβ oligomer counts in treated individuals.

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Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

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Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes in Health Research (CIHR)

Title: Real-time *In vivo* imaging reveals the ability of monocytes to clear vascular amyloid beta

Authors: *J.-P. MICHAUD, M.-A. BELLAVANCE, P. PRÉFONTAINE, S. RIVEST; CHUL Res. Ctr., Sainte-Foy, QC, Canada

Abstract: Cerebral amyloid angiopathy has a prevalence of more than 90% in patients with Alzheimer’s disease (AD) and correlates with cognitive deficits. Although the contribution of monocytes has yet been restricted of being macrophage precursors in AD, we aimed to investigate whether monocytes could play a role in the elimination of vascular Amyloid Beta (Aβ). By live intravital two-photon microscopy, we demonstrate that monocytes are attracted and crawl onto Aβ-positive blood vessels of APPswe/PS1 mice. Furthermore, we report the presence of crawling monocytes carrying Aβ and their ability to circulate back into the bloodstream. These observations uncover the ability of monocytes to naturally eliminate vascular Aβ and constitute a potential new therapeutic target in AD.

Disclosures: J. Michaud: None. **M. Bellavance:** None. **P. Préfontaine:** None. **S. Rivest:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.02/J17

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: Analysis of TREM2 expression in APPPS1 transgenic mouse brain

Authors: A. ARRANZ[1,2], *B. G. DE STROOPER[3,2];
[1]VIB Ctr. for the Biol. of Dis., Leuven, Belgium; [2]Ctr. for Human Genet. and LIND, KU Leuven, Leuven, Belgium; [3]K.U.Leuven, Leuven, Belgium

Abstract:
Rare variants in the TREM2 (triggering receptor expressed on myeloid cells 2) gene are associated with an increased risk of late-onset
Alzheimer disease (AD).
TREM2 is an immune phagocytic receptor expressed on the cell membrane of immature dendritic cells, osteoclasts, tissue macrophages and brain microglia. TREM2 on microglia is critical for the clearance of neuronal debris and has an anti-inflammatory role in the brain. Therefore, impaired function of the TREM2 protein may affect inflammatory processes and lead to a decline in cognitive function through the
inability of the brain to clear amyloid plaques.
In mouse models of AD, TREM2 is expressed in microglia surrounding
amyloid plaques however full characterization of TREM2 expression in these mouse models is lacking. Therefore, by using qPCR, immunohistochemistry and western blotting we have analyzed the expression of TREM2 gene and protein in APPPS1 transgenic mice and we have compared it with their control littermates.
Our results might provide insight into how TREM2 impairment leads to the CNS symptoms of AD.

Disclosures: A. Arranz: None. **B.G. De Strooper:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.03/J18

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: ARDRAF

Title: IL-1β mRNA expression, circadian rhythms, and nest building in an hAPP/ApoE4 late-onset mouse model of Alzheimer’s disease

Authors: *P. BOZZELLI, J. J. GRAYBEAL, L. L. GRAYBEAL, D. N. COX, J. M. FLINN; George Mason Univ., Fairfax, VA

Abstract: Inflammation is one of the hallmarks of Alzheimer's disease (AD). The majority of AD studies of inflammation use mouse models carrying genes associated with early-onset AD. These early-onset genes however only account for about 5% of AD cases. In order to study the more prevalent form of AD we crossed animals carrying an early-onset gene (APP) with a late-onset gene (ApoE4). Recent evidence suggests that in spite of conferring a significant risk of developing AD later in life, the ApoE4 gene may be protective in young mice (Marchant et al., 2010). Behavioral assays were conducted at 4 months and mice were then sacrificed at 7 months. RT-PCR was performed to evaluate gene expression for the inflammatory cytokine IL-1β. Mice carrying the single transgene APP had significantly higher expression of IL-1β compared to wildtype controls (Mann-Whitney U test, $p<.001$). The APP/ApoE4 mice were not significantly different from wildtype controls (Mann-Whitney U test, $p<.201$). The levels of IL-1β gene expression were then correlated with wheel-running activity variables, which are a measure of circadian rhythm. Significant correlations were observed in average bout length ($r=.509$, $p<.05$), average number of counts per bout ($r=.573$, $p<.05$), average peak rate during a bout ($r=-.509$, $p<.05$), and the average number of counts per bout-minute ($r=-.647$, $p<.01$). A nest building assay was also performed by introducing a cotton square into the wheel-running cages during the last 48 hours of circadian rhythm testing. Wildtype mice built nests while APP mice did not. IL-1β expression levels were significantly correlated with scores of nest building ($r=-.812$, $p<.000$). The APP/ApoE4 mice showed intermediate ability in constructing nests which parallels their intermediate IL-1β levels. These data indicate that ApoE4 status may be protective against disruptions in circadian rhythms, nest building, and cytokine levels in young mice.

Disclosures: P. Bozzelli: None. **J.J. Graybeal:** None. **L.L. Graybeal:** None. **D.N. Cox:** None. **J.M. Flinn:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.04/K1

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH AG023084

NIH NS034467

Title: Human RAGE expressed in endothelial cells exacerbates blood-brain barrier dysfunction

Authors: *S. V. REGE[1], E. HATCH[2], M. WANG[1], N. K. CHUQUI[1], Z. ZHAO[1], A. P. SAGARE[1], B. V. ZLOKOVIC[1,2];

[1]Physiol. and Biophysics, USC, Los Angeles, CA; [2]Ctr. for Neurodegenerative and Vascular Brain Disorders, Univ. of Rochester, Rochester, NY

Abstract: The receptor for advanced glycation end products (RAGE) is responsible for the recognition of a diverse array of ligands and this interaction activates the receptor resulting in an inflammatory response. RAGE is present mainly at the luminal, or blood-facing side, of the brain endothelium at the blood-brain barrier (BBB) and mediates the influx of amyloid β-peptide (Aβ), Alzheimer's disease neurotoxin, into the brain parenchyma. Altered activity of RAGE in the BBB endothelium may contribute to the Aβ accumulation leading to uncoupling of cerebral blood flow and metabolism, neuronal injury, as well as inflammation. In diabetes, hyperglycemia stimulates the production of ligands that interact with RAGE and activate inflammatory signaling mechanisms. To study the influence of RAGE in vascular pathology at the BBB, we have generated a transgenic mouse model overexpressing human RAGE (hRAGE) in endothelial cells under the control of the Tie-2 promoter. The expression of hRAGE was confirmed by quantative real-time PCR, Western blotting, immunohistochemistry, and mass spectrometry analysis. We observed age-dependent changes in basement membrane and tight junction proteins, decreased pericyte coverage and BBB breakdown associated with accumulation neurotoxic/vasculotoxic serum proteins in brain of hRAGE expressing mice. The transgenic expression of hRAGE in vascular endothelial cells leads to these pathological changes in the BBB by 14 months of age. Thus, this model can be used to determine the RAGE mediated progression of vascular pathology associated with hyperglycemia, specifically at the BBB. In addition, newly developed RAGE inhibitors by our group (J Clin Invest. 2012; 122(4):1377-1392) could be used with this model as a rescue for the observed pathology.

Disclosures: S.V. Rege: None. **E. Hatch:** None. **M. Wang:** None. **N.K. Chuqui:** None. **Z. Zhao:** None. **A.P. Sagare:** None. **B.V. Zlokovic:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.05/K2

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: FIS-PI12/01431 (to AG)

FIS-PI12/01439 (to JV)

CIBERNED grant PI2010/08 (to AG and JV)

Title: Microglial responses to disease progression in human Alzheimer hippocampus

Authors: *A. GUTIERREZ[1], E. SANCHEZ-MEJIAS[1], V. NAVARRO[2], R. SANCHEZ-VARO[1], S. JIMENEZ[2], L. TRUJILLO-ESTRADA[1], M. VIZUETE[2], J.-C. DAVILA[1], J. VITORICA[2];
[1]Univ. of Malaga /CIBERNED, Malaga, Spain; [2]Univ. of Seville / CIBERNED / IBIS, Seville, Spain

Abstract: We have previously reported the temporal progression of microglial activation in the hippocampus of a PS1/APP transgenic model of Alzheimrer's disease, however the microglial phenotypes in the human pathology have not been determined yet. We have here characterized the microglial response during disease progression using hippocampal samples from human autopsies of middle-age controls, Braak II, Braak III-IV and Braak V-VI Alzheimer patients using RT-PCR, western blots, immunostainings and cell systems.
Clear microglial activation was identified on BraakV-VI samples. Using the same cohort, we tested the microglial differentiation state (M1 or M2) by determining the expression of several key factors. No changes in the expression of the murine alternative marker Arg-1 was observed. However, we detected a slight decrease in of, at least, three putative human alternative markers (mannose-receptor 1, IGF-1 and folate-receptor-2). In the same samples, the classic markers TNF-alpha, FASL and CH3CL1 displayed a clear increase. This cytotoxic inflammatory environment is coincident with a significant decline of the neuronal marker NeuN. Interestingly, a profound reduction in the expression of SOM, NPY and PV interneuronal markers was found. Furthermore, we have also tested the stimulatory capacity, in vitro using microglial cultures, of soluble extracts (S1 fractions), prepared from the same cohort of human samples. Using these preparations, the S1 derived from Braak V-VI samples produced a M1 stage whereas the S1 from Braak II induced a M2 polarization.
In conclusion, similar to animal models, aged non-demented human samples displayed a non-classic partially-alternative microglial polarization whereas demented Alzheimer samples displayed a more pronounced classic activation. We are now characterizing the human derived soluble S1 fractions trying to identify the putative mediators of the microglial activation.

Disclosures: A. Gutierrez: None. **E. Sanchez-Mejias:** None. **V. Navarro:** None. **R. Sanchez-Varo:** None. **S. Jimenez:** None. **L. Trujillo-Estrada:** None. **M. Vizuete:** None. **J. Davila:** None. **J. Vitorica:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.06/K3

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: CIRM grant to DWE (RN1-00538)

Title: Prominent loss of adaptive immune responses to Aβ in perimenopausal women who carry an apoe-ε4 allele

Authors: *A. N. BEGUM[1], C. CUNHA[1], E. R. ROSARIO[2], J. SCOLNICK[3], D. W. ETHELL[1]; [1]Mol. Neurobio. Group, Col. of Biomed. Sci., Western Univ. of Hlth. Sci., Pomona, CA; [2]Casa Colina Hosp. for Rehabilitative Med., Pomona, CA; [3]Neurosurg., Arrowhead Regional Med. Ctr., Colton, CA

Abstract: Alzheimer's disease (AD) is the most common causes of dementia in the elderly, with >5.2 million cases in the US, a number that will exceed 13 million by 2030. Disappointing results in recent clinical trials that targeted immune responses to Aβ has shifted strategies toward slowing AD progression at the earliest stages possible. Identifying subjects in the early prodromal stages of this disease currently rely on measures that require at least some pathological changes in the brain. Our lab has taken a different approach by evaluating adaptive immune responses to Aβ that may be useful as early indicators of changes in the handling of Aβ as it is cleared from the brain. To achieve this goal we developed a novel platform that allows for the characterization of Aβ-specific CD4+ T cell responses using a small sample of human blood, referred to as CD4see. Notably, the immune responses evaluated by CD4see are similar, if not identical, to those elicited by Aβ vaccination. Aβ vaccination attenuates AD pathology and cognitive impairment in mice, suggesting a protective role for Aβ-specific CD4+ T cells. CD4see employs stem cell-derived dendritic cells (DC) that are engineered to present highly specific fragments of Aβ to CD4+ T cells isolated from whole blood, using HLA fusion proteins that span Aβ1-42. Blood samples that contain CD4+ T cells showed T cell proliferation in response to one or more of these probes, which is detected by flow cytometry. In our analysis of >75 human subjects we found that most young and middle-aged people have Aβ-specific CD4+ T cells, demonstrating a capacity for an adaptive immune response to Aβ. Alternatively, Alzheimer's subjects had few Aβ-specific CD4+ T cells. Women who carried at least one copy of the ApoE-ε4 allele showed few Aβ-responsive CD4+ T cells over the age of 52, which is a perimenopausal period for most women. Interestingly, these women are at very high risk for AD with a more rapid progression of dementia. Age-dependent reductions of Aβ-specific CD4+ T cell responses were not as distinct in men who carried an ApoE-ε4 allele, or in non-carriers of both genders. CD4see may provide an important early measure of changes in adaptive immune responses to Aβ that could aid in identifying subjects in the prodromal stages of AD, which could be beneficial in clinical trials that target Aβ-specific immune responses to treat AD.

Disclosures: A.N. Begum: None. **C. Cunha:** None. **E.R. Rosario:** None. **J. Scolnick:** None. **D.W. Ethell:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.07/K4

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH grant P20GM103486

NIH grant NS079637

Title: Determining the effects of neuroinflammatory phenotypes on amyloid deposition in APP/PS1 transgenic mice

Authors: *E. M. WEEKMAN, T. L. SUDDUTH, A. GREENSTEIN, D. M. WILCOCK; Physiol., Univ. of Kentucky, Lexington, KY

Abstract: The polarization of neuroinflammatory phenotypes has been described in early Alzheimer's Disease (AD), yet the impact of these different phenotypes on the pathology of AD remains unknown. The goal of the current study was to determine whether an M1 neuroinflammatory phenotype affects amyloid pathology in a different manner than an M2a phenotype. To address this we injected Adeno-Associated Virus serotype 8 (AAV-8) expressing either IL-4 or TNFα and IFNγ into the frontal cortex and hippocampus of both the right and left hemispheres of the brain in wildtype and APP transgenic mice. Mice receiving IL-4 AAV-8 were sacrificed at 4 to 6 weeks due to mortality. Mice injected with TNFα and IFNγ AAV-8 were sacrificed at 4 months or 6 months. The neuroinflammatory phenotype and microglial activation were measured by qPCR and immunohistochemistry, respectively.

We found that AAV-8 expressing IL-4 led to an M2a biased neuroinflammatory phenotype while TNFα and IFNγ led to an M1 biased phenotype. This was determined by qPCR for the M1 markers IL-1β, TNFα, IL-6 and IL-12 and the M2a markers Ym-1, IL-10 and IL-1Ra. Microglial activation assessed by CD11b and CD45 showed increased activation with both IL-4 and TNFα/IFNγ. However, it was noted that plaque-associated microglial activation was more intense in the mice receiving TNFα/IFNγ AAV-8. Amyloid-beta quantification is currently in progress.

Overall, we found that IL-4 AAV-8 promotes an M2a phenotype while TNFα and IFNγ AAV-8 promotes an M1 phenotype. Also, microglial activation showed a higher degree of plaque association in mice receiving TNFα and IFNγ AAV-8.

Disclosures: E.M. Weekman: None. **T.L. Sudduth:** None. **A. Greenstein:** None. **D.M. Wilcock:** None.

Poster

238. Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.08/K5

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: National Institute on Aging R15AG033913

Title: MyD88 and the inflammasome are both involved in the activation of microglia by amyloid-β(1-42) protofibrils

Authors: *S. TERRILL, M. MOHAN, M. R. NICHOLS;
Univ. of Missouri St. Louis, Saint Louis, MO

Abstract: Neuroinflammation is a well-defined element of Alzheimer's disease (AD). The brains' of AD patients contain senile plaques composed of aggregated amyloid-β protein (Aβ). Surrounding these plaques are activated microglia which secrete a variety of proinflammatory cytokines and create an inflammatory environment involving both innate and adaptive immune responses in the central nervous system. Data has shown that several multi-protein receptor complexes mediate the microglial immune response to Aβ and suggest that multiple innate immune components work together for Aβ recognition. Previous work in our lab has identified Toll like receptors (TLRs) as having a role in mediating Aβ-induced inflammatory responses. The activation of TLRs leads to the recruitment of the TLR adaptor protein MyD88 and the expression of many different innate immune genes. We have previously shown that Aβ(1-42) protofibrils are potent activators of primary murine microglia. Therefore we utilized primary microglia from both wild type (WT) and MyD88 knockout ($MyD88^{-/-}$) mice to investigate the involvement of MyD88 in the Aβ-induced inflammatory response. Our data shows that WT primary microglia treated with Aβ(1-42) protofibrils significantly increased mRNA and protein levels of tumor necrosis factor alpha (TNFα) and interleukin-1beta (IL-1β) compared to buffer controls. $MyD88^{-/-}$ primary microglia treated with protofibrils produced <1% of the WT primary microglia TNFα and IL-1β mRNA after two hours. However, while secreted TNFα protein for $MyD88^{-/-}$ microglia was <20% of the WT microglia, secreted IL-1β protein was observed at 1 hour of treatment and did not show a decrease through 6 hours. Furthermore, the IL-1β protein response was comparable between WT and $MyD88^{-/-}$ microglia at all-time points suggesting that reduction of IL-1β mRNA by knockdown of MyD88 does not diminish mature IL-1β protein production. The findings herein demonstrate that the TLR-MyD88 pathway plays a significant role in mediating the Aβ(1-42) protofibril-induced proinflammatory response but is not

necessarily linked to protofibril activation of the inflammasome.

Disclosures: S. Terrill: None. **M. Mohan:** None. **M.R. Nichols:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.09/K6

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: FONDECYT 1131025

Title: Participation of Scavenger Receptor A (SR-A) in the inflammatory activation pattern of glial cells in an Alzheimer's disease new transgenic model

Authors: *R. VON BERNHARDI, F. CORNEJO, G. RAMIREZ;
Dept Neurol, Pontificia Univ. Catolica De Chile, Fac Med., Santiago, Chile

Abstract: Scavenger receptors appear to participate in β-amyloid (Aβ) uptake and the inflammatory activation of glia. We propose that binding to specific ligands can mediate neuroinflammatory processes that are characteristic in neurodegenerative diseases. In APP/PS1 transgenic mice, we observed that young animals had an increased percentage of astrocytes and microglia expressing SR-A compared with WT mice. However, the number of cells was reestablished at 6 months of age. We developed a triple-transgenic mice overexpressing Aβ and KO for SR-A (APP/PS1/SR-$A^{-/-}$), in which we observed an increased production of Nitric Oxide (NO) by microglia, and a decreased release of IL1β by astrocytes and microglia in response to inflammatory stimuli such as LPS, whereas a reduction of TNFα release was observed in stimulated astrocytes. Furthermore, triple-transgenic astrocytes showed an increased phagocytosis of Aβ, whereas microglia showed an apparent reduction in the induction of phagocytic activity in response to LPS. Trimeric SR-A receptor was expressed by WT astrocytes, whether KO cells only expressed the monomeric protein, which is not functional. We also evaluated the participation of SR-A on astrocytes and microglial cell activation as well as on the ability of astrocytes to regulate microglia. Astrocytes regulate neuroinflammation through the production of several soluble molecules, including IL1β, which is capable of regulating microglial cell activation. In astrocyte cultures obtained from WT and SR-$A^{-/-}$ mice, we analyzed the activation of MAPKs and IκB/NFκB signaling pathways and the production of NO, TNFα and IL1β in response to SR-A ligands (LPS and Poly I). LPS was capable of inducing activation

of ERK in WT and KO cells, but activation lasted longer in KO astrocytes. In WT but not KO cells, LPS induced phosphorylation of JNK and p38. Similarly, IκB/NFκB pathway was active both in WT and SR-A$^{-/-}$ cells, but activation of IκB/NFκB was delayed in KO astrocytes. LPS increased production of NO in a time dependent manner, but levels of NO were higher in KO than in WT astrocytes. In contrast, whereas SR-A ligands increased TNF-α release in WT and KO astrocytes, IL1β release was undetectable in stimulated KO cells. Our results suggest that SR-A participates in several phenomena associated to Alzheimer's disease, such as the activation of signaling pathways involved in the production of soluble molecules mediating the inflammatory activation of glial cells, and is needed to modulate neuroinflammatory cell activation.

Disclosures: R. von Bernhardi: None. **G. Ramirez:** None. **F. Cornejo:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.10/K7

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: CIHR Operating Grant

Centre for Studies on Prevention of Alzheimer's Disease (StoP-AD Centre)

Title: Early increase in TNF-alpha in a prodromal model of Alzheimer's disease

Authors: *C. CAVANAGH, R. QUIRION, T. WONG;
Psychiatry, Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada

Abstract: An enormous effort is directed at targeting amyloid-beta (Abeta), the main constituent of senile plaques, to treat Alzheimer's disease (AD). Unfortunately, all clinical trials with this approach have failed, leading to alternative hypotheses of causative agents in AD. The focus has also shifted to the prodromal stages of the disease to identify the earliest alterations contributing to AD in hopes to improve intervention. Inflammatory mediators, such as pro-inflammatory cytokines, are present from early to late stages of AD pathology and can have neurotoxic effects. For example, tumor necrosis factor-alpha (TNF-alpha) is upregulated in both humans with AD and transgenic mouse models of the disease. So far, inhibition of TNF-alpha signaling has been beneficial in mouse models (Gabbita et al., J. Neuroinflammation, 2012) and has even led to cognitive improvement in an AD patient (Tobinick et al., J. Neuroinflammation, 2008). Despite these studies, the mechanism by which TNF-alpha may exacerbate AD pathology is unknown,

however, it is possible that TNF-alpha is affecting synaptic function. Although TNF-alpha is a well-known immune mediator, increasing data indicates that TNF-alpha has functions in the brain as well. Here, the role of TNF-alpha is thought to be regulatory or neuroprotective at physiological levels, however at increased levels, TNF-alpha may become neurotoxic (Santello and Volterra, Trends in Neurosci., 2012, Cavanagh et al., Future Neurology, 2011). We hypothesize that increased levels of TNF-alpha at early stages of AD-like pathology may become pathogenic and lead to deficiencies in synaptic function. We use the TgCRND8 mouse model of AD, which overexpresses a double human mutation of the precursor to Abeta, the amyloid precursor protein (APP) to show that 1-month-old mice display both a significant increase in TNF-alpha (Cavanagh et al., J. Alzheimer's disease, 2013) and alterations in synaptic function. This time point is well suited for the study of prodromal AD, since it occurs before the development of cognitive deficits and the appearance of amyloid plaques. Our findings may provide insight on TNF-alpha as a potential therapeutic target in the prodromal stages of AD and will help elucidate the effects of TNF-alpha on synaptic function.

Disclosures: C. Cavanagh: None. **R. Quirion:** None. **T. Wong:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.11/K8

Topic: C.03. Alzheimer's Disease and Other Dementias

Title: An acute lps-induced inflammatory response in a diabetic model of alzheimer's disease

Authors: *A. S. MURTISHAW[1], C. F. HEANEY[2], J. W. KINNEY[2], M. M. BOLTON[2];
[1]Psychology/Neuroscience, UNLV Neurosci. Doctoral Student, Las Vegas, NV;
[2]Psychology/Neuroscience, UNLV, Las Vegas, NV

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder of unknown etiology. AD is characterized by cognitive and behavioral impairments in addition to pathological features that include amyloid plaques, neurofibrillary tangles and neuronal loss. Only a small proportion of AD cases are due to genetic mutations whereas the vast majority of cases are late onset and sporadic in origin. The cause of sporadic AD (sAD) is likely multifactorial, with interactions of external factors, biological, and genetic susceptibilities that contribute to the onset and and progression of the disease. Diabetes Mellitus (DM) and neuroinflammation are two of the most common risk factors that have been implicated in sAD. Considerable progress has been made to understand the involvement of each of these risk factors in isolation but limited data exist on the

combination of the two. In the below studies we investigated the effects of neuroinflammation in a diabetic-model of sAD on behavioral and pathological markers of AD. For the present study, we infused streptozotocin (STZ; a compound used to model sAD in animals) directly into the lateral ventricles to dysregulate insulin signaling within the brain. This initial investigation was directed at determining the effects of an acute inflammatory response on STZ-induced deficits relevant to AD. Lipopolysaccharide (LPS) was utilized to acutely activate the immune system one week following the STZ infusion. Learning and memory was examined in the Morris water maze (MWM), following which hippocampal tissue was examined for pathological markers of AD. Results indicate that STZ-induced deficits consistent with other reports in both behavioral tasks as well as an increase in oligomeric beta-amyloid consistent with AD. Interestingly, the acute inflammatory response significantly reduced both the behavioral deficits and the increase in oligomeric beta-amyloid. These data demonstrate a beneficial effect of an acute inflammatory response in this model (STZ+LPS).

Disclosures: A.S. Murtishaw: None. **C.F. Heaney:** None. **J.W. Kinney:** None. **M.M. Bolton:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.12/K9

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: CIHR Grant MOP89728

Wings for Life Grant WFL-CA-005/12

Title: Epigenetic regulation of aging microglia in Alzheimer’s Disease and neurodegeneration

Authors: *E. M. YORK[1,2,4], A. PETIT[1,4], A. CHOU[3], A. J. ROSKAMS[1,4];
[1]Zoology, [2]Neurosci., [3]Cell. and Physiological Sci., Univ. of British Columbia, Vancouver, BC, Canada; [4]Intl. Collaboration On Repair Discoveries, Vancouver, BC, Canada

Abstract: In a healthy brain, microglia are neuroprotective; however with age, they become senescent and lose their regulative, proteolytic, and neuroprotective functions. Dystrophic microglia appear in the Alzheimer’s disease (AD) brain before tau pathology becomes apparent and may be present in other forms of cognitive dementia. We are interested in whether chromatin dysregulation contributes to the senescence of microglia, making them inefficient at performing their normal scavenging function in the aging brain. Aging of peripheral immune cells can be

directly modulated by histone deacetylase inhibitors (HDACis). HDACis can also shift glia and neurons into a more developmental state to promote or aid CNS repair, and alter microglia activation and macrophage recruitment after spinal cord injury.
We hypothesize that aging, cellular damage, and epigenetic factors cause microglia to become dystrophic, which decreases their ability to neuro-protect and respond to injury. This facilitates more long-term damage and progression of neurodegenerative disease. We have found that dystrophic microglia - identified morphologically and by enhanced expression of L-ferritin - are more numerous in the human AD neocortex compared to controls. In addition, we have observed that microglia are dysregulated with aging in the PSEN mouse model of AD. In our experiments, HDACis do appear to alter the mitotic abilities of both young and aged primary microglia cultures from adult mice in vitro, and we are currently testing if this is reflected in morphology and ability to respond to stimuli, in vitro. If so, this, coupled with approaches to produce and better characterize dystrophic microglia in vitro, will allow us to assess the ability of HDACis to revert a dystrophic microglia phenotype to that of a younger cell. We will then screen for changes in the expression and activity of individual HDACs so that highly targeted HDACis in microglia can be tested to limit off-target effects. These results will allow us to test if dystrophic microglia may malfunction as part of aging and neurodegenerative disease or neurodegeneration after injury, and if HDAC inhibition may be an appropriate approach to restore their protective functions. These results will be important in understanding and treating brain injury across the lifespan, and in developing novel approaches to mobilize microglia in the context of neurodegenerative diseases.

Disclosures: E.M. York: None. **A. Petit:** None. **A. Chou:** None. **A.J. Roskams:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.13/K10

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Mount Sinai Alzheimer Disease Research Center ((Dr. S Gandy, Dr. P Hof, and Dr. M Sano, PI; Drs. O Bozdagi Gunal and JD Buxbaum) U01 P50 AG005138-28

Title: Characterization of caspase-4 in inflammation and Alzheimer's disease

Authors: Y. KAJIWARA, N. DORR, G. VOLOUDAKIS, M. GAMA-SOSA, G. ELDER, D. L. DICKSTEIN, *O. BOZDAGI, J. D. BUXBAUM;
Dept Psychiatry, Icahn Sch. of Med. At Mount Sinai, NEW YORK, NY

Abstract: Caspase-4 has been proposed to be a primate-specific member of the inflammatory caspases. We have previously reported that caspase-4 mRNA is significantly upregulated in the brain of Alzheimer's disease (AD) subjects. However, due to the absence of the gene in the mouse genome, physiological function of caspase-4 has remained unknown. We therefore generated transgenic mice expressing human caspase-4 and characterized them in the context of innate immune response and AD pathogenesis.

Consistent with its inflammatory role, in response to lipopolysaccharide caspase-4 transgenic mice rapidly secreted higher levels of proinflammatory cytokines including interleukin-1β (IL-1β) and interleukin-18 (IL-18), both of which are known substrates of caspase-1. In addition, bone marrow derived macrophages from caspase-4 mouse secrete IL -1β and IL-18 in response to TLR ligand alone. IL-1β and IL-18 hyper-responsivity was abolished in caspase-1 deficient mice, supporting the role for caspase-4 as an upstream activator of caspase-1.

The role for caspase-4 in AD was investigated by crossing caspase-4 mice with APPsw/PS1deltaE9 mice. Caspase-4 expression was upregulated in male APPsw/PS1deltaE9/CASP4 mice and this upregulation was specific to brain regions relevant to AD. Caspase-4 did not affect either amyloid volume in the hippocampus or the level of soluble Abeta in the brain. Electrophysiological analysis showed a decrease in synaptic plasticity measured by long-term potentiation in caspase-4 mice at 14 month-old but not in 4 week-old mice. When spatial learning ability was tested by Barnes maze, all mice showed normal acquisition at 7 and 13 month. However, male APPsw/PS1deltaE9/CASP4 mice showed significant impairment upon reversal of target compared to all other genotypes at both ages.

Disclosures: Y. Kajiwara: None. **N. Dorr:** None. **G. Voloudakis:** None. **M. Gama-Sosa:** None. **G. Elder:** None. **D.L. Dickstein:** None. **O. Bozdagi:** None. **J.D. Buxbaum:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.14/K11

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Maurice Marciano Family foundation

The Coins for Alzheimers Research Trust (C.A.R.T) Fund

The National Center for Advancing Translational Sciences through CTSI Grant UL1TR000124

Title: Glatiramer acetate induces cerebral infiltration of osteopontin-expressing monocytes that facilitate Aβ clearance

Authors: *A. RENTSENDORJ[1], B. SALUMBIDES[1], J. SHEYN[1], Y. KORONYO[1], D. LOPES[1], D.-T. FUCHS[1], A. WOLF[2], K. L. BLACK[1], M. KORONYO-HAMAOUI[1,3];
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Abstract: Previously, we reported that immune modulation with glatiramer acetate (GA; also called Copaxone®) reduces neuropathology and cognitive impairments associated with Alzheimer's disease (AD) in transgenic murine models (ADtg mice). These effects were associated with cerebral recruitment of bone marrow (BM)-derived monocytes/macrophages inducing a local anti-inflammatory response, via IL-10 and MMP-9 secretion, and amyloid β-protein (Aβ) plaque clearance. However, the exact mechanism by which GA induces peripheral monocyte recruitment to the brain and alleviates AD progression in mice is still unclear. Osteopontin (OPN) is a glycoprotein highly expressed by BM-derived monocytic cells that serves as a modulator of immune cell migration, communication, and overall response, partly via induction of MMP-9 and by shifting helper T cell populations. To understand the role of OPN in GA-immunized ADtg mice and how GA affect OPN levels in myelomonocytic cells, OPN expression in GA-immunized bigenic APPSWE/PS1dE9 mice was studied. Further, the extent by which GA impacts OPN expression in macrophage and their phagocytic capacity to uptake fibrillar Aβ, were evaluated in primary BM-derived cultures. We found distinctive elevated OPN expression patterns in different brain regions, such as entorhinal and cingulate cortex and hippocampus, following GA immunization. A combined treatment of GA with i.v. infusion of BM-monocytes (GABM) indicated even higher OPN levels surrounding Aβ plaque sites with increased monocytic infiltration. OPN colocalized with and around Iba1+CD45high macrophages that were associated with the plaques. Correlogram analysis of quantitative immunohistochemical data from these brain regions indicated a significant linear association between OPN and infiltrating monocytes/macrophages, as well as inverse correlation between OPN levels and Aβ plaque burden. In vitro analyses, using western blot, ELISA and immunocytochemical assays, revealed that GA modulates OPN expression in BM-macrophages concomitant to enhanced cellular uptake of fibrillar Aβ1-42. These in vitro results mirror our in vivo data reinforcing the notion that OPN mediates migration of BM-monocytes to the diseased brain and affect macrophage behavior. Overall, these studies suggest that reduced cerebral Aβ burden achieved by GA immunization is mediated at least in part through increased recruitment of OPN-secreting monocytes/macrophages with enhanced ability to clear Aβ.

Disclosures: A. Rentsendorj: None. **B. Salumbides:** None. **J. Sheyn:** None. **Y. Koronyo:** None. **D. Lopes:** None. **D. Fuchs:** None. **A. Wolf:** None. **K.L. Black:** None. **M. Koronyo-Hamaoui:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.15/K12

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIA grant R01 AG34103

Title: Diet-induced obesity and low testosterone interact to promote Alzheimer- and diabetes-related pathologies: A central role of inflammation

Authors: *J.-W. LEE[1], H. WRIGHT[2], C. J. PIKE[1];
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Abstract: Obesity, type 2 diabetes (T2D) and low testosterone are common health issues in aging men and independent risk factors for Alzheimer's disease (AD). Epidemiological studies suggest bidirectional associations between these factors, linking low testosterone with increased risk of obesity and T2D and vice versa. In this study, we investigated whether these factors interact in regulating AD development and, if so, what pathway(s) contribute to the interaction. To accomplish this goal, we compared the effects of diet-induced obesity in male mice with normal and low levels of testosterone on metabolic and Alzheimer-related endpoints. Since chronic inflammation is a common feature in AD, obesity, and T2D, we also examined neural and peripheral metabolic inflammatory responses as a potential contributing pathway to hypothesized interactions. Specifically, young adult male, C57BL/6J mice were maintained for four months on diets containing either 10%, 45%, or 60% fat. Separate groups of mice under each diet had normal testosterone, low testosterone (orchiectomy, ORX) or ORX with testosterone treatment. At the end of the treatment period, mice were behaviorally assessed and various tissues were collected and analyzed for levels of factors associated with AD, T2D, and inflammation. We observed that high fat diet resulted in significantly increased bodyweight, fat mass, and indicators of T2D including fasting glucose and insulin levels. Obese mice also exhibited significant increases in soluble β-amyloid protein and decreased expression of the β-amyloid degrading enzyme neprilysin. Further, obese mice exhibited increased levels in brain and adipose tissues of pro-inflammatory genes such as TNFα, IL-1β and IL-6. Each of these outcomes was exacerbated in ORX mice. Importantly, the deleterious effects of ORX were prevented by testosterone treatment. Low testosterone even in the absence of high fat diet had negative effects on expression of metabolic, AD, and inflammatory factors. This study demonstrates independent and interactive effects of low testosterone and obesity on indices of AD and T2D. Notably, low testosterone and obesity also cooperatively promoted inflammatory pathways, which are implicated in the pathogenesis of both AD and T2D, suggesting that

inflammatory responses may be a central part in this interrelationship. These findings demonstrate significant beneficial roles of testosterone in neuronal and metabolic conditions that may provide insight into effective therapeutic strategies for AD and metabolic disorders.

Disclosures: J. Lee: None. **H. Wright:** None. **C.J. Pike:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.16/L1

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Fondos Mixtos CONACYT-Queretaro 2012

Title: Role of oxidative status, metabolic syndrome, and associated factors in neurodegenerative diseases with a higher prevalence, of central Mexico State

Authors: J. G. GONZALEZ-LOYOLA[1], J. C. SOLÍS-S[1], P. GARCÍA-SOLÍS[1], L. ROBLES-OSORIO[1], N. CAMACHO-CALDERON[1], J. AVILA-MORALES[1], M. E. VILLAGRAN-HERRERA[1], B. E. CASTRO-MONTES[1], F. J. DAVILA-ESQUIVEL[1], G. Z. LELO DE LARREA[1], G. GONZALEZ-PEREZ[1], M. LIÑAN-FERNANDEZ[1], J. RICARDO-GARCELL[2], M. RAMOS-GOMEZ[3], M. ESPINO-CORTES[4], M. E. C. CETINA-GARCIA[4], L. M. VELAZQUEZ-PEREZ[4], L. GARCIA-GALVEZ[4], M. L. MARTINEZ-MARTINEZ[5], R. F. RODRIGUEZ-VALDEZ[6], *H. L. HERNANDEZ MONTIEL[1];
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Abstract: Most just over 6,000 million people who inhabit the planet today have a longer life expectancy than did their counterparts half a century ago and these, in turn, much larger than their ancestors had done tens of thousands of years ago. In our species is seated a biological tendency consisting of the gradual increase of life and are, therefore, a propensity to undergo degenerative processes themselves senescence. Parkinson's disease (PD) and Alzheimer's disease (AD) is the most prevalent neurodegeneration contemporary human societies and have started to become a high impact epidemiological problem by the progressive deterioration of motor and cognitive skills that characterize them. In the world literature there are many mentions of association of a number of signs that determine metabolic syndrome (MetS) with the degenerative processes mentioned, but still difficult to make categorical statements of a cause-

effect relationship. The alarming increase in the incidence and prevalence of overweight/obesity, MetS and diabetes mellitus in the world's population, can presume to observe any link with the increased prevalence of neurodegenerative diseases. Target. To determine the presence of oxidative stress-related factors and metabolic syndrome in patients with PD and AD. Methods. We compared three groups of elderly patients drawn from the general population who come to consult to Neurodiagnostics Unit and Nervous System Diseases Research, Faculty of Medicine, Universidad Autonoma of Queretaro: the first group consists of individuals diagnosed with PD, the second by individuals diagnosed with AD, and the third by individuals without such neurodegeneration or demonstrated signs of MetS. All three groups are different biochemical procedures are applied (curve glucose tolerance, glycosylated hemoglobin, fasting serum glucose, insulin determination and calculation of insulin resistance), neuropsychological performance (test for depression, drug addiction, alcoholism neurocognitive status), neurodiagnostic studies (conventional and quantitative electroencephalography, visual and auditory evoked potentials) and are being applied a questionnaire to determine your lifestyle (exercise, diet). The criteria for defining the PD, AD and MetS variables were the established by the World Health Organization. Results. At this time, partial results have different procedures. State conclusions. Because it is an ongoing investigation, the findings and results will be presented at the meeting in San Diego, CA.

Disclosures: J.G. Gonzalez-Loyola: None. **H.L. Hernandez Montiel:** None. **J.C. Solís-S:** None. **P. García-Solís:** None. **L. Robles-Osorio:** None. **N. Camacho-Calderon:** None. **J. Avila-Morales:** None. **M.E. Villagran-Herrera:** None. **B.E. Castro-Montes:** None. **F.J. Davila-Esquivel:** None. **G.Z. Lelo de Larrea:** None. **G. Gonzalez-Perez:** None. **M. Liñan-Fernandez:** None. **J. Ricardo-Garcell:** None. **M. Ramos-Gomez:** None. **M. Espino-Cortes:** None. **M.E.C. Cetina-Garcia:** None. **L.M. Velazquez-Perez:** None. **L. Garcia-Galvez:** None. **M.L. Martinez-Martinez:** None. **R.F. Rodriguez-Valdez:** None.

Poster

238. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 238.17/L2

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG030399

NIH Grant AG037814

Title: Effects of TLR4 overexpression and deficiency on neuropathology in a mouse model of Alzheimer's disease

Authors: J. KOU[1], J. YANG[1], J. D. FLORES[2], D. MALO[3], *K.-I. FUKUCHI[1];
[1]Cancer Biololgy and Pharmacol., [2]Pediatrics, Univ. of IL Col. of Med. At Peoria, Peoria, IL;
[3]Medicine; Human Genet., McGill Univ., Montreal, QC, Canada

Abstract: The accumulation of amyloid β-protein (Aβ) in the brain is postulated to be a primary etiologic event leading to dementia of Alzheimer's disease (AD). Fibrillar Aβ deposits are accompanied by activated microglia and increased levels of cytokines. Many lines of evidence support the notion that activated microglia, innate immune cells in the central nervous system, play pivotal, dual roles in AD progression: either clearing Aβ deposits by phagocytosis and promoting neuron survival and plasticity or releasing cytotoxic chemicals, inflammatory cytokines, exacerbating Aβ load and neurodegeneration. Aβ aggregates/deposits can activate microglia through a receptor complex that contains toll-like receptor 4 (TLR4) as one of its essential components. To investigate the role of TLR4 signaling in the pathogenesis and progression of AD, we have produced three groups of AD model mice, which differed in TLR4 copy number: 1) 6 copies of the TLR4 gene (overexpression), 2) 1 copy, and 3) no copy (TLR4-deficient). These model mice were produced by repeated mating between Tg390 mice carrying 6 copies of the TLR4 gene on the C57BL/10ScNCr background and TgAPPswe/PS1dE9 mice on the C57BL/6J background. TLR4 overexpression (6 copies) and deficiency did not influence Aβ load in the brain of an AD mouse model at 5 months of age. We are currently investigating the effects of TLR4 overexpression and deficiency on β-amyloidosis and neuroinflammation in these animal models at 10 months of age.

Disclosures: J. Kou: None. **K. Fukuchi:** None. **J. Yang:** None. **J.D. Flores:** None. **D. Malo:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.01/L3

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG020569

NIH Grant AG028367

Title: A role for thrombin as a mediator of cerebrovascular abnormalities in Alzheimer's disease and diabetes

Authors: *P. GRAMMAS, A. SANCHEZ, J. LUO, X. YIN;
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Abstract: Alzheimer's disease (AD) and diabetes are two of the most common and devastating health problems in the elderly. Epidemiological evidence indicates that diabetes is associated with an increased risk for the development of AD. Vascular abnormalities are important features of both AD and diabetes, thus the brain vasculature is a likely nexus for the convergence of diabetic and AD pathogenic mechanisms. In the cerebromicrovasculature we have previously demonstrated oxidative injury as well as elevated expression of numerous inflammatory/neurotoxic proteins, including thrombin and amyloid beta. In the periphery, high glucose, a critical factor in the development and progression of diabetic complications, evokes vascular oxidative stress To explore the effects of high glucose on brain endothelial cell function, cultured brain-derived endothelial cells, with and without high glucose pretreatment (25 mM, 48 h), were exposed to oxidative stress induced by H2O2 (0.5 mM), menadione (25 µM), or SNP (5 mM). Thrombin activity was measured, levels of thrombin and amyloid beta quantitated by ELISA, and indices of cell injury determined by MTT assay and staining for caspase 3 and superoxide. The results indicated that high glucose pretreatment potentiated oxidant-mediated increase in levels of thrombin and thrombin activity as well as Abeta levels. Oxidative stress-induced indices of cell injury were all exacerbated in cultures exposed to high glucose, compared to cultures not treated with glucose. Treatment of cultured endothelial cell cultures with the thrombin inhibitor dabigatran diminished glucose-induced increase in thrombin, Abeta, caspase 3 and superoxide. Finally, examination of transgenic AD mice made diabetic by treatment with streptozotocin (STZ) showed that AD+STZ mice expressed higher cerebrovascular levels of thrombin compared to non-diabetic AD mice. Taken together these data suggest that glucose-induced microvascular injury may be mediated, in part, by thrombin and that therapeutic targeting of this inflammatory protein may hold promise in mitigating cerebrovascular abnormalities in both in AD and diabetes.

Disclosures: P. Grammas: None. **A. Sanchez:** None. **J. Luo:** None. **X. Yin:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.02/L4

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: JSPS Grant 25460150

Title: Intracerebral microinjection of interleukin-4/interleukin-13 reduces β-amyloid accumulation in the ipsilateral side and improves cognitive deficits in young APP23 mice

Authors: *K. KAWAHARA, M. SUENOBU, Y. SUGIMOTO, H. NAKAYAMA;
Fac. of Life Sci. Kumamoto Univ., Kumamoto, Japan

Abstract: We previously reported that the anti-inflammatory cytokine interleukin (IL)-4 induced selective clearance of oligomeric β-amyloid (Aβ1-42) in rat primary type 2 microglial cells. For the present study, we investigated whether IL-4 and IL-13 could activate microglial cells to induce Aβ clearance in vivo and improve cognitive deficits in APP23 mice, which are amyloid precursor protein transgenic mice. We administered an intracerebral microinjection of a mixture of IL-4 and IL-13 or of saline vehicle into one hemisphere of APP23 mice and their wild-type littermates, 4.5 and 9 months old, after which we evaluated the effects of these treatments on spatial learning and memory by Morris Water Maze test and on accumulated amounts of Aβ. The cytokine injection significantly improved memory deficits of 4.5-month-old APP23 mice, but did not do so in 9-month-old APP23 mice, even though similar Aβ reductions were observed in both age groups of APP23 mice in the ipsilateral neocortex. The cytokine injection improved memory impairment of 9-month-old WT mice in the probe trial. Immunohistochemical analysis of the 4.5-month-old APP23 mice revealed the presence of increased numbers of microglial cells at 2 days after the cytokine injection. In addition to induced CD36 expression in the activated microglia, increased expression of neprilysin, mainly in neurons, suggested that the cytokines improved the cognitive deficits via degradation and clearance of intra- and extraneuronal Aβ peptides, of buffer-extractable nonplaque form. Double immunostaining also revealed that most of the activated microglia had the M2-like phenotype. This unique mechanism of IL-4/IL-13-induced clearance of Aβ may provide an additional strategy to prevent and/or cure Alzheimer's disease at early stage.

Disclosures: K. Kawahara: None. **M. Suenobu:** None. **Y. Sugimoto:** None. **H. Nakayama:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

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Program#/Poster#: 239.03/L5

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Coins for Alzheimer's Research (CART)

NIH Grant AG030482

Title: Nuclear receptor agonists increase Aβ mediated microglial phagocytosis and influence microglial activation status in a mouse model of AD

Authors: *J. SAVAGE, G. E. LANDRETH;
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Abstract: Alzheimer's Disease is a neurodegenerative disease characterized by accumulation of amyloid beta (Aβ) within the brain and appearance of fibrillar amyloid beta (fAβ) plaques. These plaques arise from the imbalance of production and clearance of Aβ. Microglia migrate to and invest processes into fAβ plaques. This interaction leads to microglial activation, expression of proinflammatory cytokines, and reactive oxygen species. The chronic inflammatory response within the AD brain renders microglia incapable of phagocytosing and degrading fAβ. Other tissue-specific macrophages are capable of resolving inflammation, repairing damaged tissue, and phagocytosing cellular debris by converting to an "alternatively activated" M2 state. We hypothesize that conversion of microglia to an M2 state could be of benefit in AD due to their anti-inflammatory and phagocytic capabilities. However, M2 activation in microglia has not been well studied.
Activation of the peroxisome proliferator-activated receptors (PPARs) and liver x receptors (LXRs) drive M2 macrophage polarization and enhance phagocytosis. Treating AD model mice with LXR, PPAR, or retinoid X receptor (RXR) agonists improves cognition and decreases plaque load. The loss of Aβ plaques is coincident with the appearance of phagocytically active, amyloid-laden microglia. However, the mechanism of fAβ clearance has not been established. We hypothesized that treatment with nuclear receptor agonists drives M2 polarization of microglia and increases phagocytosis and degradation of fAβ. We report that treatment of the microglial cell line N9 with synthetic agonists of RXRs (bexarotene) increases their phagocytic capacity, and treatment of primary microglia with bexarotene increases expression of phagocytic receptors MerTK and Axl. Bexarotene treatment of microglia also suppresses TNF, iNOS, and Cox2 expression in response to LPS treatment. Bexarotene treatment of 5XFAD mice decreases plaque burden, and increases microglial expression of the phagocytic machinery genes Thbs1, Ly9, and clec4e. Surprisingly, bexarotene treatment increases microglial expression of both proinflammatory cytokines including IL-1 and IL-6 and anti-inflammatory cytokines and markers including IL-10, arginase, and IL1R2. These data show nuclear receptor agonist treatment of AD animals causes functional changes in microglial phenotype, including increased phagocytosis of amyloid.

Disclosures: J. Savage: None. **G.E. Landreth:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.04/L6

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG030482

Gail & Elliott Schlang Philanthropic Fund

NIH Grant 5T32NS067431-13

CAPES Foundation Grant 5758/12-2

Title: LXR and PPARγ agonists modify inflammation differentially in a mouse model of Alzheimer's disease

Authors: *R. SKERRETT[1], M. P. PELLEGRINO[2], G. E. LANDRETH[1];
[1]Neurosciences, Case Western Reserve Univ., Cleveland, OH; [2]Sch. of Med., Univ. of Campinas, Campinas, Brazil

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a decreased ability to clear amyloid β from the brain. Previous work has shown that this clearance is linked to the cholesterol carrier Apolipoprotein E (Apo E) and its lipidating proteins ABCA1 and ABCG1. The expression of these proteins is regulated by the transcription factor liver X receptor (LXR) and, through a complex metabolic pathway, with peroxisome proliferator-activated receptor (PPARγ). Our studies show that treatment of aged APPswe/PS1dE9 mice with either an LXR or PPARγ agonist results in increased protein expression of ABCA1, ABCG1, and ApoE, a reduction in soluble species of amyloid β as well as in cortical plaque load, and improved performance in the contextual fear conditioning test. Activation of LXR and PPARγ can also decrease inflammatory gene expression by transrepression of inflammatory genes at NFκB promoters, and PPARγ is involved in modulating the activation state of microglia into an "alternative" activation state. Modifying harmful neuroinflammation, which is a characteristic of AD, could therefore be a further benefit of LXR and PPARγ agonists. In vitro treatments of microglia with LXR agonists, PPARγ agonists, or a combination of both results in activation of different sets of genes. Alternative activation genes such as arg1, ym1, fizz1 and TGFβ are differentially regulated by each treatment. However, treating with LXR and PPARγ agonists together appears to reduce the inflammatory markers IL6, TNFα and IL1β most efficiently. Combination therapy with these agonists might therefore be therapeutically valuable in an AD model. In vivo, 6 month old mice treated jointly with LXR and PPARγ agonists show a reduction in inflammatory markers in the brain similar to that achieved in mice treated with either LXR or PPARγ agonists alone. Regulation of genes

associated with alternative activation widely varied between treatments. However, mice treated by combination therapy showed diminished amyloid burden and behavioral performance improvements similar to those of mice treated with LXR or PPARγ agonists separately. Nuclear receptor regulation of microglial phenotype is a complex process that merits further study.

Disclosures: R. Skerrett: None. **M.P. Pellegrino:** None. **G.E. Landreth:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.05/L7

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Delaware Bioscience Center for Advanced Technology

NSF ECS-9876771

Title: Utilization of an *In vitro* model of the blood-brain barrier to study a potential Alzheimer's disease therapeutic

Authors: *D. WUEST, K. H. LEE;
Univ. of Delaware/ Delaware Biotech Inst., Newark, DE

Abstract: Numerous immunotherapeutic approaches to Alzheimer's disease (AD) have targeted the amyloid-β peptide, and the passive immunization strategy, intravenous immunoglobulin (IVIG), has been identified to contain anti-amyloid-β antibodies as well as antibodies that provide immunomodulatory effects to reduce the negative consequences of chronic inflammation in AD. Mechanisms of action of IVIG are likely multifaceted and complex, and because IVIG is currently under investigation for AD in clinical trials, a deeper understanding of how IVIG works in AD is highly valuable. Important questions about IVIG transport into the central nervous system and any impact on this transport as a function of amyloid-β may be best provided with the use of in vitro models of the blood-brain barrier (BBB).
The development of in vitro experimental approaches to study the cellular constituents and the dynamic capabilities of the BBB typically rely on the growth of a monolayer of cerebral endothelial cells grown on a porous membrane submerged in the wells of a multi-well plate, allowing numerous conditions to be tested simultaneously. With its ability to exhibit transcellular and paracellular diffusion pathways, a cell-based model offers the potential to account for metabolism and active transport processes. The murine model we use has been developed and optimized to demonstrate growth of uninterrupted endothelial cell monolayers for appropriate

transport analysis.
Here, we investigate BBB phenomena occurring in AD and its treatment with IVIG therapy. Specifically, we studied the effect and transport capability of IVIG on the BBB pre-incubated with amyloid-β and the immunomodulatory effect of IVIG on cytokines released by astrocytes in response to amyloid-β. Amyloid-β[1-42], was pre-aggregated into fibrillar or oligomeric structures, and various concentrations were incubated in the abluminal side. IVIG was administered to the luminal side at different concentrations, and IgG, the principal constituent of IVIG, and astrocyte-derived cytokines were quantified in the abluminal side in all incubation combinations of amyloid-β structure, amyloid-β concentration, and IVIG dose. IVIG accumulated in the abluminal side at physiologically relevant levels, with more accumulation resulting from amyloid-β pre-incubation, and IVIG was found to affect cytokine levels. Our investigation into the transport and immunomodulatory effect of IVIG therapy across the BBB in an AD cellular model revealed a correlation of BBB loosening and inflammation in response to amyloid-β and insight into effects of IVIG in AD.

Disclosures: D. Wuest: None. **K.H. Lee:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.06/L8

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH grant NS-21182

Title: Chronic lipopolysaccharide increases neuroinflammation, reduces plaque pathology and induces cognitive deficits in a mouse model of alzheimer’s disease

Authors: *D. Y. LO[1,2], P. E. SAWCHENKO[1];
[1]Salk Inst., La Jolla, CA; [2]Biol., Coe Col., Cedar Rapids, IA

Abstract: Accumulating evidence supports a role for inflammation in the pathogenesis of Alzheimer’s Disease (AD). Peripheral infectious/inflammatory challenges can impact the inflammatory state of the brain and accelerate disease onset and/or progression. The underlying pathways by which systemic inflammation impact the progression and severity of AD are undefined, and the effects of systemic inflammation over the course of AD have not been characterized. Addressing these issues may lead to improved therapeutic management of AD. We have begun to investigate the effects of systemic inflammation on neuropathological processes in AD, and have undertaken parallel behavioral analyses in order to relate

neuropathological changes to deficits in cognitive function. Transgenic mice that express mutant human amyloid precursor protein (APP) and presenilin 1 (PS1) and their wild-type littermates were treated twice weekly with lipopolysaccharide (LPS; 0.5 mg/kg, ip) or saline for 6 weeks prior to sacrifice at 6, 10 or 14 months of age. Quantitative analysis of amyloid pathology through amyloid immunolabeling (4G8 antibody) revealed that LPS treatment resulted in a significant decrease in cortical, but not hippocampal, plaque load in cohorts of PS1/APP mice sacrificed at 14 months. LPS treatment also significantly increased the number of cyclooxygenase (COX-2)-immunoreactive endothelial cells, a marker of vascular inflammatory activation, in 14-month-old animals compared to the 6- and 10-month-old PS1/APP mice. Morphological indices of LPS-induced microglial activation (Iba-1 staining) were also reliably elevated in 14-, relative to 6- and 10-, month-old PS1/APP mice. To associate our histochemical findings with alterations in cognitive function, mice were tested on the Barnes and Y-mazes. Assessing performance through measures of latency and number of errors, performance on the Barnes maze revealed LPS treatment-induced deficits in learning and memory in 14-month-old PS1/APP mice compared to wild-type saline controls. Spontaneous alternation performance in the Y-maze showed similar deficits in 14-month-old PS1/APP mice treated with LPS. Overall our results show that chronic inflammatory challenge exaggerates CNS inflammation in aged AD mice, which is associated with decreased amyloid plaque pathology and deficits in cognitive function. These results support a role for inflammatory activity in limiting beta amyloid deposition. However, systemic inflammation has a net effect of worsening AD outcome, emphasizing a need to better understand the mechanisms of intercommunication between the immune and central nervous systems that mediate these effects.

Disclosures: D.Y. Lo: None. **P.E. Sawchenko:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.07/L9

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: ZEN-11-202716

Title: The role of TLR4 signaling in the mechanism of action of bexarotene for the treatment of Alzheimer’s disease pathology

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Abstract: Alzheimer's disease is associated with an increase in soluble Aβ in the brain, the deposition of fibrillar forms of Aβ (fAβ) into plaques, and cognitive dysfunction. In addition, this pathology is associated with an altered neuroinflammatory state. TLR4 is an important innate immune receptor and, along with its coreceptors CD14 and CD36, is an essential element of the fAβ receptor complex. Signaling through this receptor induces a pro-inflammatory reaction in microglia. Recent research has shown that the RXR agonist, bexarotene, stimulates the clearance of soluble and insoluble Aβ and improves cognitive deficits in aged APP/PS1 mice. We explored the role of microglia in bexarotene-stimulated Aβ clearance by genetically inactivating the TLR4 signaling pathway. The APP/PS1 mouse model of amyloidosis was crossed with mice that had a loss-of-function mutation of TLR4 (TLR4M Tg). TLR4M Tg mice show increased levels of soluble Aβ, increased plaque deposition, and increased cognitive impairment when compared with APP/PS1 with mice functional TLR4 receptors (TLR4WT Tg). Bexarotene treatment or vehicle was given for 8 days by oral gavage (100 mg/kg/day) to TLR4M or TLR4WT mice. Fear conditioning was performed the last 2 days of treatment, after sacrifice, half of the brain was processed for protein and Aβ analyses. Bexarotene caused a significant increase in the RXR target genes ABCA1, ABCG1, and ApoE in TLR4WT Tg mice, but only ApoE was significantly increased by bexarotene in TLR4M Tg mice. Bexarotene treatment enhanced learning in tone-based fear conditioning in the TLR4WT Tg mice, but not in TLR4M Tg mice. Levels of soluble and insoluble Aβ were determined in the cortex by ELISA. TLR4M Tg mice showed a decrease in soluble Aβ, but no change in insoluble Aβ following bexarotene treatment. In comparison, TLR4WT Tg mice showed a reduction in both soluble and insoluble Aβ. To determine neuroinflammatory changes in the brain, a panel of inflammatory markers were measured by qPCR in the cortex. The neuroinflammatory profile of the cortex was changed by bexarotene in both TLR4M and WT Tg mice, but in non-identical ways. These data indicate that TLR4 signaling is necessary for the ability of bexarotene to stimulate insoluble Aβ clearance, but not soluble Aβ clearance. In addition, functional TLR4 signaling is necessary for the cognitive benefits of bexarotene treatment.

Disclosures: A.W. Corona: None. **M.M. Lakner:** None. **K. Fukuchi:** None. **G.E. Landreth:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.08/L10

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: The Texas Higher Education Controlling Board- Norman Hackerman Advanced Research Program (to MJC)

Title: The effects of poly i:c on hippocampal amyloid-beta and cognition

Authors: *M. K. WEINTRAUB[1], D. KRANJAC[1], M. J. EIMERBRINK[1], B. T. VINSON[2], J. PATEL[2], W. SUMMERS[2], B. WOMBLE[1], G. W. BOEHM[1], M. J. CHUMLEY[2];
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Abstract: Alzheimer's disease (AD) currently affects 5.4 million Americans, and is the 6th leading cause of death in the United States. AD is a progressive disorder characterized by neurodegeneration in regions of the adult brain, including the hippocampus and cortex. The two hallmark pathologies of AD are amyloid-beta (Aβ) plaques and neurofibrillary tangles, and presence of these pathologies can limit cell-to-cell communication ultimately leading to learning and memory deficits. Although A plaques were originally thought to cause the detrimental effects in the brain, more simple forms of Aβ, such as monomers, dimers, tetramers and oligomers, have also been shown to be potentially neurotoxic. Chronic inflammation has been implicated in the onset and progression of these AD pathologies. Our lab has previously shown that peripheral injections of a bacterial mimetic led to increased Aβ levels in the mouse hippocampus, as well deficits in learning (Kahn et al., 2012). The current study was designed to further our understanding of peripheral inflammation-induced AD-like pathology by using polyinosinic:polycytidylic acid (poly I:C), which induces inflammation similar to that induced by double-stranded viral RNA. Mice were given intraperitoneal injections of poly I:C or saline once a day for seven consecutive days. Similar to our findings using the bacterial mimetic LPS, hippocampal tissue from animals receiving poly I:C also contained significantly higher levels of Aβ, a peptide component of AD plaques. Interestingly, we determined that animals required 48 hours following the 7th injection of poly I:C to no longer experience sickness behaviors. This was unlike LPS treatment, after which animals became tolerant to the LPS treatments midway through the injection series and no longer displayed sickness behaviors following the final LPS injection. Similar to our finding when using LPS, animals administered poly I:C also displayed significant cognitive deficits in the hippocampus-dependent contextual fear conditioning paradigm, extending our hypothesis that increased levels of Aβ can lead to cognitive deficits even when the Aβ has not aggregated into plaques.

Disclosures: M.K. Weintraub: None. **D. Kranjac:** None. **M.J. Eimerbrink:** None. **B.T. Vinson:** None. **J. Patel:** None. **W. Summers:** None. **B. Womble:** None. **G.W. Boehm:** None. **M.J. Chumley:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.09/L11

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH NIA 5R00AG029726-04

NIH NIA 3R00AG029726-04S1

NIH NINDS 1R01NS076794-01

Alzheimer’s Association Zenith Fellows Award (ZEN-10-174633)

American Federation of Aging Research/Ellison, and Medical Foundation Julie Martin Mid-Career Award in Aging Research (M11472)

Title: Network analysis of transcriptional changes after two anti-inflammatory pathway manipulations in the PSAPP mouse reveals Alzheimer disease neuroinflammatory targets

Authors: *K. R. DOTY[1], M.-V. GUILLOT-SESTIER[1], D. GATE[1,2], T. TOWN[1];
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Abstract: Alzheimer’s disease (AD) is characterized by extracellular deposition of amyloid-β (Aβ) peptides into β-amyloid plaques and neuronal injury and loss. In addition to these hallmark AD pathological features, neuroinflammation is established early and maintained chronically in brains of affected individuals. The etiology of brain inflammation, its role in disease progression, and the possibility of targeting beneficial forms of neuroinflammation to prevent AD initiation or promote regression have remained elusive. As the resident innate immune cells of the brain, microglia become activated in close vicinity of β-amyloid plaques. However, these mononuclear phagocytes are ineffective at clearing Aβ and preventing consequent buildup of β-amyloid plaques. While the reasons for microglial dysfunction in AD are unknown, one approach to correct this imbalance is to modulate neuroinflammation. Recently, our laboratory has knocked out two anti-inflammatory pathways in the PSAPP mouse model of cerebral amyloidosis. Promoting inflammation via genetic deletion of interleukin-10 or endorsing toll-like receptor signaling by knocking out the natural dominant-negative, interleukin-1 receptor associated kinase-M (IRAK-M), leads to mitigation of cerebral amyloidosis in vivo. We hypothesize that specific inflammatory mediators, as opposed to a general inflammatory state, are responsible for

the reduced AD-like pathology that we have observed. Using next-generation sequencing (RNAseq) and network analysis, we have uncovered differentially expressed genes in common to both genetic manipulations. Identification of these differentially expressed genes has allowed us to make predictions regarding unique neuroinflammatory signaling pathways that could be targeted to mitigate cerebral amyloidosis.

Disclosures: K.R. Doty: None. **M. Guillot-Sestier:** None. **D. Gate:** None. **T. Town:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.10/L12

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Regeneron Pharmaceuticals

Title: Chronic systemic inhibition of interleukin-1 reduces spatial learning deficits in a mouse model of Alzheimer's disease without affecting amyloid plaque burden

Authors: *S. N. RESCH[1,2], J. LUTCHMAN[3], J. JOHNSON[1], A. ROE[3], T. MOY[2], K. SINGH[3], A. PEREZ[1], C. NAVAS[3], S. D. CROLL[1,2,3];
[1]Neuropsychology Doctoral Subprogram, The Grad. Ctr. of CUNY, New York, NY; [2]Regeneron Pharmaceuticals, Tarrytown, NY; [3]Psychology, CUNY Queens Col., Flushing, NY

Abstract: Chronic neuroinflammation, which includes elevated expression of the cytokine interleukin-1 (IL-1), is a prominent feature of Alzheimer’s disease and has been hypothesized to contribute to the neurodegeneration and cognitive dysfunction observed in patients with the disease. In this study, we used systemic administration of Regeneron's mouse IL-1 (mIL-1) Trap to inhibit interleukin-1 signaling in the swAPP-PS1 double transgenic mouse model of Alzheimer's Disease. Wild type and transgenic mice were chronically treated with either an Fc control protein or mIL-1 Trap (10mg/kg s.c. 2x/wk) starting at approximately 8 months of age to assess the effect of these reagents on hippocampally-dependent spatial learning and memory as well as Alzheimer’s-like brain pathology. During the fifth month of treatment, animals were tested in the Morris Water Maze task, in which they received three consecutive trials per trial block for two blocks per day across five days. Median latency to escape to the platform per trial block was used as a measure of acquisition. At the end of the fifth day, animals were placed back into the water maze for a 30 second spatial probe trial. Retention was measured using both the amount of time the animals spent in the goal quadrant and the number of crosses over the location of the goal platform. Animals were sacrificed 5 months after the initiation of treatment

and brains were removed and analyzed for amyloid plaque burden using Congo Red staining. As expected, the double transgenic mice showed significantly worse water maze acquisition than their wild type controls, and the mIL-1 Trap significantly improved acquisition in the double transgenic mice. In contrast, there was no significant effect on retention of the platform location during the spatial probe trial in the transgenic mice, suggesting the possibility of the use of compensatory strategies for performing the task in the treated animals. Amyloid plaque burden in the double transgenics was not significantly altered by administration of mIL-1 Trap. However, mIL-1 Trap decreased the positive relationship between plaque burden and cognitive impairment measured in the Fc-treated transgenics, suggesting that although the plaques were present, they were no longer associated with cognitive impairments in the animals. We are currently in the process of studying the effect of chronic systemic mIL-1 Trap treatment on other cells in the brain, with a special focus on cells most likely to have been exposed to systemic mIL-1 Trap such as the endothelial cells and subpopulations of microglial-like cells.

Disclosures: S.N. Resch: None. **J. Lutchman:** None. **J. Johnson:** None. **A. Roe:** None. **T. Moy:** None. **K. Singh:** None. **A. Perez:** None. **C. Navas:** None. **S.D. Croll:** A. Employment/Salary (full or part-time):; Regeneron Pharmaceuticals. 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.; Regeneron Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Regeneron Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.11/L13

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association NIRG-12-242598

NIH R00AR054695

Title: Upregulation of glutamate transporter 1 (GLT1) by ceftriaxone ameliorates Alzheimer’s disease-associated cognitive impairment in 3xTg-AD mice

Authors: *J. M. ZUMKEHR[1], R. MEDEIROS[2], D. CHENG[2], Y. YANG[3], M. KITAZAWA[4]; [1]Mol. and Cell Biol., UC Merced, Merced, CA; [2]Neurobio. and Behavior, Univ. of California,

Irvine, Irvine, CA; [3]Neurosci., Tufts Univ., Boston, MA; [4]Mol. and Cell Biol., Univ. of California, Merced, Merced, CA

Abstract: Synaptic loss is an early pathological event in Alzheimer's disease (AD) and correlates well with the severity of cognitive decline in patients with AD as well as mild cognitive impairment. Although exact molecular and cellular mechanisms of synaptic loss have not been elucidated in AD, excitotoxicity is speculated to be one of the major mechanisms that trigger synaptic degeneration. To support this, it has been reported that high glutamate levels are found in cerebrospinal fluid from AD patients, and a loss of astrocytic glutamate transporters correlates with synaptic loss in AD. In addition, reduced glutamate transporter 1 (GLT1) expression in a mouse model of AD shows an early and accelerated cognitive deficit. GLT1 or its human ortholog Excitatory Amino Acid Transporter 2 (EAAT2) prevents the over stimulation of the post synapse by removing 90% of glutamate levels from the synaptic cleft. Therefore, we hypothesized that a loss of GLT1/EAAT2 is one of the early pathological changes in AD and mediates synaptic degeneration and cognitive decline associated with AD neuropathology. We found that fibrillar amyloid-beta (Aβ) reduced GLT1 levels in primary astrocyte culture. In 3xTg-AD mice, GLT1 expression was significantly decreased with age and a buildup of AD-like pathology in the brain. We next pharmacologically upregulated GLT1 using ceftriaxone, a beta lactam antibiotic known to up regulate GLT1, in 3xTg-AD mice. Two-month treatment with ceftriaxone significantly ameliorated a cognitive decline in 3xTg-AD mice. Synaptic density was restored by the treatment as well. Interestingly, both Aβ burden and tau pathology were not significantly altered by the ceftriaxone treatment in 3xTg-AD mice, suggesting that a beneficial effect on cognition was mainly mediated by a restoration of synaptic density. Together, these results show promising evidence that a functional upregulation of GLT1 plays an important role in delaying the synaptic degeneration and cognitive decline in AD.

Disclosures: J.M. Zumkehr: None. **R. Medeiros:** None. **D. Cheng:** None. **Y. Yang:** None. **M. Kitazawa:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.12/L14

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: American Health Assistance Foundation

Title: Role of the pro-inflammatory transcription factor, NFATc2, in regulating Aβ stimulated microgliosis

Authors: *A. GHATAK, K. L. PUIG, C. K. COMBS;
Pharmacology, Physiol. & Therapeut., Univ. of North Dakota, Grand Forks, ND

Abstract: Alzheimer's disease (AD) is characterized by formation of amyloid β (Aβ) peptide containing plaques and neurofibrillary tangles in the brain. Reactive microglia, the resident phagocytes of the brain, are often hypothesized to be interacting with Aβ. Our prior work has demonstrated a role for the transcription factor, NFAT (Nuclear Factor of Activated T Cells) in regulating microglial activation. In this study we hypothesized that NFAT activity would be particularly important for the phenotype change stimulated by microglia-Aβ interaction. Using primary mouse cultures we observed that NFATc2 is the most abundantly expressed isoform in microglia. Moreover, NFATc2 levels were highest in microglia compared to neuron and astrocyte cultures. Consistent with this expression pattern, Aβ 1-42 stimulation of microglia increased activity of NFATc2, but not NFATc1. Inhibition of NFAT activity with a cell permeable inhibitory peptide, 11R-VIVIT, or the small molecule, FK506, attenuated the Aβ stimulated increase in TNFα and IL-6 secretion by microglia. To validate a role for particularly NFATc2 in regulating Aβ stimulated activation, microglia from NFATc2 knockout and wild type mice were compared. Both Aβ and the bacterial endotoxin, lipopolysaccharide, had decreased ability to stimulate TNFα and IL-6 secretion from NFATc2 knockout microglia compared to controls. In addition, both 11R-VIVIT and FK506 demonstrated attenuated ability to inhibit Aβ-stimulated cytokine secretion in the NFATc2 knockout microglia compared to controls. These data demonstrated that NFATc2 activity was required for Aβ stimulated activation. Importantly, intraperitoneal injection of the 11R-VIVIT peptide was sufficient to inhibit NFATc2 activity in the brains and spleens of wild type mice. However, NFATc1 activity was inhibited in spleen but not in brain. This suggests that brain selective inhibition of NFATc2 activity is feasible. In order to begin identifying a relevant AD mouse model for assessing the role of NFAT-mediated microgliosis *in vivo*, we examined 12 month old male transgenic (APP/PS1) mouse brains to observe elevated brain protein levels of NFATc2 compared to wild type male C57BL/6 mice. This was consistent with the increased microglial activation characteristic of the AD model suggesting that these mice will be useful for examining NFAT-mediated inflammation during disease. As a whole, our findings indicate the possibility of developing anti-inflammatory drugs for AD that selectively target the proinflammatory transcription factor, NFATc2.

Disclosures: A. Ghatak: None. **K.L. Puig:** None. **C.K. Combs:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.13/L15

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Swiss National Science Foundation grant number 310030–132629

University of Zurich Candoc Forschungskredit

Rahn & Bodmer, Private Banking, Zurich

Title: Reelin immunoreactivity in neuritic varicosities in the human hippocampus

Authors: *T. F. NOTTER, I. KNUESEL;
Univ. of Zurich Inst. of Pharmacol. and Toxicology, Zurich, Switzerland

Abstract: By activating its receptors ApoER2/VLDL, Reelin modulates actin and microtubule cytoskeleton dynamics, thereby regulating neuronal migration/positioning during development and glutamatergic synaptic plasticity in cortical regions in the adult brain. Age-dependent reduction in Reelin levels has been suggested to contribute to Alzheimer's disease (AD) pathophysiology by increasing amyloid precursor protein (APP) proteolytic processing and Tau phosphorylation, as well as by altering NMDA receptor activation and their synaptic availability via an ApoE-dependent mechanism. We have previously reported a reduction in Reelin levels in aged mice and its accumulation in neuritic varicosities in olfactory and limbic system pathways, which correlated with cognitive impairments. Here, we aimed to investigate whether a similar Reelin-associated neuropathology is observed in aged human hippocampal formation and whether it is correlated with dementia. Using 8 specimens of AD patients and 8 of non-demented age-matched controls provided by the Netherland Brain Bank, we observed strong immunoreactivity for N- and C-terminus-containing Reelin fragments in corpora amylacea (CAm), spherical glycoprotein inclusions associated with aging and chronic neurological diseases. Stereological analysis showed that the density of these deposits was increased in the molecular layer of the subiculum of AD patients compared to non-demented individuals. Further, we detected several neuronal but no astrocytic/microglial markers co-localized with Reelin-immunoreactivity in CAm, suggestive of aging-associated impairments in neuronal transport machinery, leading to formation of neuritic varicosities and CAm. Therefore, the presence of Reelin in CAm provides a novel indicator for aging and AD-related neuronal damage and degeneration.

Disclosures: T.F. Notter: None. **I. Knuesel:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.14/L16

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: NIH R01 CA123194

UNM Brain and Behavior Health Institute

The NM Fraternal Order of Eagles

Title: Reduction of Alzheimer's disease related neuroinflammation induced by trans-stillbenes

Authors: *N. O. SOLBERG[1], J. VIGIL[2], V. SEVERNS[3], L. O. SILLERUD[2];
[1]UCSD, San Diego, CA; [2]Biochem. and Mol. Biol., [3]Pathology, Univ. of New Mexico, Albuquerque, NM

Abstract: Alzheimer's disease is associated with a microglia-dependent neuroinflammatory response against plaques containing the fibrous protein B-amyloid. Activation of microglia, which closely associate with amyloid plaques, engenders the release of pro-inflammatory cytokines and the microglia mediated internalization of B-amyloid plaque. Since the pro-inflammatory transcription factor NF-kB is one of the major regulators of B-amyloid induced inflammation, we treated transgenic Amyloid Precursor Protein/Presenilin-1 (APP/PS1) mice with either of two *trans*-stilbene analogs, resveratrol or its synthetic analog LD-55, which prior work had shown to inhibit NF-kB in vitro. Amyloid plaques were measured *ex vivo* by quantitative anti-APP conjugated SPION-enhanced MRI of intact brains and by optical microscopy of thioflavin-stained brain tissue sections. The regional distribution of microglial activation was measured *ex vivo* by Iba-1 immunofluorescence of brain tissue sections. We developed 3D numerical models of both plaque and microglial areal density distributions that describe the data. It was found that treatment with either resveratrol or LD-55 *concomitantly* decreased both B-amyloid plaque and activated microglial areal densities by 3 to 6-fold in a region dependent manner. We have concluded that both resveratrol and LD-55 are efficient at reducing B-amyloid plaque burden and are highly effective at reducing neuroinflammation *via* a reduction in number of activated microglia.

Disclosures: N.O. Solberg: None. **J. Vigil:** None. **V. Severns:** None. **L.O. Sillerud:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.15/L17

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Taipei Veteran General Hospital (Grant V101E4-002)

Title: The immune inhibition effect of DcR3 on modulating microglia activation phenotypes, neuronal plasticity and cognitive functions in Alzheimer's disease

Authors: *Y. LIU[1], I.-J. CHENG[1,2];
[1]Natl. Yang-Ming University/ Inst. of Brain Sci., Taipei, Taiwan; [2]Natl. Yang-Ming University/ Brain Res. Ctr., Taipei, Taiwan

Abstract: The complex inflammatory responses drive series of neurodegeneration and cognitive decline in the course of Alzheimer's disease (AD). Microglia is the major immune regulator in the brain. The ratio of M1/M2 subtypes can either turn to the neurotoxic or neuroprotective processing. Decoy receptor 3 (DcR3) is able to skew the macrophages differentiation into anti-inflammatory subtypes by the neutralization ability under inflammation reaction. We hypothesize that overexpressing DcR3 may reduce Aβ-induced neuroinflammation and ameliorate AD-like neuropathology *in vitro* and in APP transgenic mouse. Female pPGK-DcR3 transgene mice were crossed to male APP transgenic mice (J20 line, PDGF-APPsw,ind) to get four genotypes of mice offspring for further biological analysis at 6-month old and 12-month of age. To identify the role of DcR3 in amyloidopathology, we examined the changes in Aß level, synaptic physiology, inflammation status, microglia phenotype and histological variations in six-month old APP/DcR3 transgenic mice. To determine functional deficits in DcR3/APP double transgenic mouse, we performed behavioral tests and electrophysiology in these transgenic mice. Results showed that Aβ increased neuroinflammation and activated microglia. DcR3/APP double transgenic mouse showed decrease total Aß level than APP transgenic mice, and showed improvement on cognitive function. The immune inhibition role of DcR3 on Aß clearance, microglia phenotype changes, and functional amelioration may be a potential therapeutic target for AD-like pathogenesis.

Disclosures: Y. Liu: None. **I. Cheng:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.16/L18

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: TCVGH-HK988008

Title: The effects of estradiol on lipopolysaccharide-induced neuroinflammation in rat mixed glial cultures

Authors: *J.-Y. WANG[1], C.-L. CHEN[2], S.-Y. CHEN[1];
[1]Dept Nursing (Basic Med. Sci), Hungkuang Univ., Taichung, Taiwan; [2]Li-Shin Hosp., Tao-Yuan, Taiwan

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder. It is associated with relevant neuroinflammatory response. The microglial cells, kind of glial cells, are the resident inflammatory cells in the brain that promote brain inflammation in response to stimuli. Astrocytes, another kind of glia, also can produce mediators that involve in neuroinflammation. The processes accompany with inflammatory response are increasing in the expression of transcription factor NFκB, inducible nitric oxide synthase (iNOS) and the release of inflammatory mediators (ex. nitric oxide (NO) and cytokines). Ultimately, these events may create a vicious cycle to induce neuronal injury.
The prevalence of AD - is recognized to be higher in women - increases with age and life expectancy is constantly increasing in civilized countries. Menopause is a natural condition of female aging in which reduction in plasma levels of estrogens lead to a variety of metabolic and physiological changes. The increase in female life expectancy during the past century has meant that women now live one-third of their lives beyond cessation of their ovarian function. And the reduction of estrogens may have important influence on elder women's life. It is known that estrogens are not only involved in reproduction, but can also modulate the central nervous system (CNS). There are many studies to investigate the relationship between estrogens and AD. But the role of estrogens in neurodegeneration has not consensus. In this study, we selected to test the influence of 17β-estradiol (E2), the predominant circulating estrogen, on lipopolysaccharide (LPS)-induced neuroinflammation in mixed glial cells.The *in vitro* experiment: rat cortical mixed glial cultures will be subjected to (1) control; (2) LPS treated with 1, 10, 100, 500, 1000 ng/ml; (3) E2 treated with 10, 100 nM, 1, 10, 100 μM ; (4) pretreatment of E2 following LPS treatment; all of above were treated for 1, 3 or 5 days. Cell density and morphology will be observed by phase-contrast microscopy. Cell injury will be assessed by MTT reduction. The production of NO will be measured to estimate the inflammatory responses. The expression of NFκB and iNOS will be estimated by western blot analysis and immunohistochemical staining. Our data indicated that the cell viability significantly decreased in LPS treatment for 1 day, but the MTT reduction (% of control) was about 90 %. The accumulation of nitrite, the expression of NFκB and iNOS were significantly increasing in 1, 3 or 5 days. Pretreatment with E2 at 100 μM significantly decreased the LPS-induced NO production and iNOS expression. We suggested that E2 can attenuate the neuroinflammatory response.

Disclosures: J. Wang: None. **C. Chen:** None. **S. Chen:** None.

Poster

239. **Alzheimer's Disease: Neuroinflammation and Immune Mechanisms II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 239.17/M1

Topic: C.03. Alzheimer's Disease and Other Dementias

Support: Emory ADRC P50 AG025688

Title: Role of TNF in modulation of immune cell traffic to the CNS by peripheral inflammation in a mouse model of Alzheimer's disease

Authors: *K. P. MACPHERSON[1], M. TANSEY[2];
[1]Emory Univ., Atlanta, GA; [2]Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Epidemiological studies have found older people who use anti-inflammatory drugs regularly have significantly lower incidence of Alzheimer's disease (AD); and newly identified AD risk factors include obesity and diabetes, conditions associated with chronic low-grade systemic inflammation. Clinically, systemic infection is associated with development of dementia in elderly patients and increased cognitive decline in AD patients. These observations support the hypotheses that (1) the peripheral immune system plays an active role in AD and (2) targeting specific elements of peripheral inflammation could be useful in treating disease. In support of this, chronic peripheral administration of lipopolysaccharide (LPS) in rodent models of neuroinflammatory disease increases central markers of inflammation (including Tumor Necrosis Factor, TNF) both acutely and chronically, is associated with enhanced cognitive decline, and correlates with primed microglia. LPS is unable to penetrate the blood brain barrier (BBB), thus peripheral inflammatory signals, such as TNF, are likely important mediators of these central effects. Evidence of increased BBB permeability is present in brains of AD patients and soluble TNF is known to increase BBB permeability. Increased BBB permeability may accelerate AD-like pathology in 5xFAD mice through increased peripheral immune signaling and traffic to the CNS in conditions of chronic systemic inflammation. Targeted neutralization of soluble TNF signaling in the CNS and inhibition of TNF synthesis can ameliorate pathology induced by systemic inflammation. However, the specific role of soluble TNF in systemic circulation and its contribution to peripheral immune cell infiltration in AD pathogenesis has not been explored. To test this, we first characterized the inflammatory profile of 5xFAD mice, a transgenic (Tg) mouse with early onset of amyloid plaques, memory impairment, and neurodegeneration. In the CNS we detected increased levels of CD45 at 4 and 6 months of age

and increased CCL2 and TNF by real-time PCR at 6 months of age in 5xFAD mice versus non-Tg mice, suggesting there is immune cell activation and neuroinflammation in the 5xFAD by 4 months of age. Studies are now in progress to determine the extent to which chronic systemic inflammation promotes peripheral immune cell traffic to the CNS in 5xFAD and non-Tg mice. Our short-term goal is to evaluate the efficacy of the novel brain-permeant soluble TNF inhibitor XPro1595 in delaying or attenuating progression of cognitive deficits and AD-like pathology.

Disclosures: K.P. Macpherson: None. **M. Tansey:** F. Consulting Fees (e.g., advisory boards); Karyopharm, Angiochem. Other; ex-employee of Xencor Inc which is developing XPro for neurological indications but is not a paid consultant and does not hold significant financial stake in the company..

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.01/M2

Topic: C.04. Parkinson’s Disease

Support: UDALL Center Grant P50NS071669 (NIH/NINDS)

R01NS037948

Yerkes Base Grant OD P51OD011132 (NIH/ORIP)

Title: Dendritic spine pathology in the primary motor cortex (M1) of MPTP-treated parkinsonian monkeys

Authors: *Y. SMITH[1], R. M. VILLALBA[2], J. F. PARE[2], T. WICHMANN[1];
[1]Yerkes Res. Ctr., Udall Ctr. Excel. For Parkinson's Dis. and Dept. of Neurol., Atlanta, GA;
[2]Yerkes Resch Crt. and Udall Ctr. of Excellence for Parkinson's Disease, Emory Univ., Atlanta, GA

Abstract: Anatomical and functional studies of the mesocortical dopaminergic system have focused almost exclusively on prefrontal cortical regions. However, the primate mesocortical dopamine system extends beyond the prefrontal cortex to densely innervate the primary motor cortex (M1). In contrast to the prefrontal innervation which originates from the ventral tegmental area, a major contingent of dopaminergic fibers in the primate M1 arises from the substantia nigra pars compacta (SNc) and retrorubral area (RRA). In MPTP-treated parkinsonian monkeys, M1 corticospinal neurons decrease their firing rate and display abnormal discharge patterns,

while corticostriatal neurons are not affected (Pasquereau and Turner, 2011, Cerebral Cortex 21:1362). These functional changes are usually interpreted as secondary to altered functions of basal ganglia-thalamocortical circuits, but they may also arise from the loss of dopamine in M1 itself. Anatomical and PET imaging data in PD patients have, indeed, shown partial degeneration of the dopamine input to M1, consistent with biochemical studies showing a 30-60% decrease of dopamine levels in M1 of MPTP-treated monkeys. Findings from our laboratory have recently shown a dramatic loss of tyrosine hydroxylase (TH)-positive innervation of M1 in parkinsonian monkeys (Weinkle et al., 2011, Soc. Neurosci. Abstr. 883.2).
Dopamine loss leads to significant spine pruning in the striatum and prefrontal cortex. The goal of the present study was to determine if dopamine denervation also induces spine loss on M1 pyramidal neurons in parkinsonian monkeys. Using Golgi impregnation, we found that M1 pyramidal neurons display as much as 40-45% loss of spines on their apical dendrites in parkinsonian animals (9.75 spines/10 µm vs 5.25 spines/10 µm of dendritic length). On the other hand, no significant spine loss was found in the cingulate cortex, a neighboring cortical region that does not display any significant dopaminergic denervation in parkinsonian monkeys (10.61 spines/10 µm vs 9.88 spines /10 µm of dendritic length). Potential changes in the extent glutamatergic inputs to M1 in parkinsonian monkeys are currently under study. Our results demonstrate significant spine loss on M1 pyramidal neurons of MPTP-treated monkeys, thereby suggesting that M1 pathology may underlie parkinsonian motor features in primates.

Disclosures: Y. Smith: None. **R.M. Villalba:** None. **J.F. Pare:** None. **T. Wichmann:** None.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.02/M3

Topic: C.04. Parkinson’s Disease

Support: Udall Center Grant P50NS071669 (NIH/NINDS)

Yekes Base Grant OD P51OD011132 (NIH/ORIP)

R01062876

Title: Preferential loss of thalamostriatal over corticostriatal glutamatergic synapses in parkinsonian monkeys

Authors: *R. M. VILLALBA[1], T. WICHMANN[2], Y. SMITH[2];
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Abstract: It is well known that striatal projection neurons undergo a significant loss of dendritic spines in animal models of Parkinson's disease (PD) and in PD patients. These spines are the termination targets of glutamatergic inputs to the striatum. It remains unclear whether the apparent pruning of glutamatergic synapses affects preferentially cortical or thalamic afferents. To address this issue, we determined the extent of thalamic and cortical terminals loss in the sensorimotor striatum (i.e., postcommissural putamen) of MPTP-treated parkinsonian monkeys. Striatal tissue from 10 adult Rhesus monkeys (5 controls and 5 MPTP-treated) was used for electron microscopic studies. Three-dimensional reconstruction of synapses and unbiased stereology methods were applied to estimate the total number of asymmetric synapses formed by vGluT1- (from cortex) or vGluT2- (from thalamus) immunoreactive terminals per unit striatal tissue volume. The total volume of tissue analyzed was 1080 μm^3 in control and 1066 μm^3 in parkinsonian animals. Overall, there was a ~40% reduction in the number of asymmetric synapses (axo-dendritic + axo-spinous) in the postcommissural putamen of MPTP-treated monkeys (compared to normal animals). We found that 74% of all asymmetric axo-dendritic synapses were lost. A subsequent analysis of this axo-dendritic synaptic pruning based on the sources (ie cortex vs thalamus) of the presynaptic terminals revealed that 80% of axo-dendritic asymmetric synapses formed by vGluT2-positive terminals and ~16% of axo-dendritic synapses formed by vGluT1-positive terminals were lost in parkinsonian animals. On the other hand, 26% of total asymmetric axo-spinous synapses were lost in MPTP-treated monkeys. Further analysis based on their vGluT content showed that the total number of asymmetric axo-spinous synapses formed by vGluT2-positive terminals was decreased by 29%, while no significant effect was noticed for the vGluT1-immunoreactive terminals. These results suggest that most of the glutamatergic denervation of the sensorimotor striatum in MPTP-treated parkinsonian monkeys is accounted for by a loss of thalamostriatal inputs, with much less degeneration of the corticostriatal system. These findings are consistent with the significant degeneration of thalamostriatal neurons in the caudal intralaminar thalamic complex, centre median/parafascicular (CM/Pf) described in PD patients and MPTP-treated monkeys (Henderson et al., 2000, Ann. Neurol. 47:345; Villalba et al., 2013, BSF, doi:10.1007/s00429-013-0507-9).

Disclosures: R.M. Villalba: None. **T. Wichmann:** None. **Y. Smith:** None.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

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Program#/Poster#: 240.03/M4

Topic: C.04. Parkinson's Disease

Support: NS057198

EB00790

EB009118

Title: Quantification of neuronal calcium transients *In vivo* in a mouse model of neurodegeneration using FLIM

Authors: *Y. YANG[1], H. UHLIROVA[2], S. L. GRATIY[2], P. A. SAISAN[3], L. REZNICHENKO[3], E. S. NORHEIM[4,3], Q. CHENG[3], K. L. WELDY[3], S. SAKADŽIĆ[5], J. M. CONNER[3], J. CUI[3], T. GONZÁLEZ[3], D. A. BOAS[5], G. T. EINEVOLL[4], A. M. DALE[2,3], E. MASLIAH[3], A. DEVOR[2,3,5];
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Abstract: In healthy neurons, multiple mechanisms ensure fast restoration of calcium concentration in the cytosol following dynamic signaling events. In brain disease, however, calcium homeostasis can be disrupted resulting in catastrophic downstream effects on the integrity of neuronal networks. Recently, using conventional (fluorescence intensity) 2-photon calcium imaging in vivo in the mouse model of neurodegeneration overexpressing the human variant of synaptic protein α-synuclein (1), we demonstrated long-lasting neuronal calcium transients characterized by considerable deviation from the exponential decay in the presence of normal spiking (2). The most evident pathology was observed in response to a repetitive stimulation when subsequent stimuli were presented before relaxation of calcium signal to the baseline. A mechanistic interpretation of these findings, however, requires knowledge of the absolute intracellular calcium concentration: at the baseline and during dynamic signaling events. To this end, we have refined an existing technology, Fluorescent Lifetime Imaging Microscopy (FLIM), to enable fast and accurate estimation of intracellular calcium concentration in vivo with 2-photon resolution and applied the technology to quantify departures from normal calcium homeostasis in the α-synuclein mouse. FLIM has been utilized previously for estimation of calcium concentration in vivo and in vitro (3, 4). We took this technology a step further by introducing a fast acquisition, sufficient for resolving the time-course of dynamic neuronal calcium changes during spontaneous activity and in response to a weak electrical stimulation of the whisker pad. Our pilot results show that, in contrast to findings in APP mouse model of Alzheimer's disease (5), the baseline calcium concentration in cortical neurons in the α-synuclein mouse is normal. Further, the amount of calcium entry during the rising phase of the response to a single-shock stimulus in the α-synuclein mouse is not significantly elevated. Taken together,

these results argue in favor of specificity of the α-synuclein model and support our original hypothesis that α-synuclein promotes alterations in calcium dynamics via interference with intracellular buffering mechanisms.
1. E. Rockenstein et al., J Neurosci Res 68, 568 (Jun 1, 2002).
2. L. Reznichenko et al., J Neurosci 32, 9992 (Jul 18, 2012).
3. C. D. Wilms et al., Cell Calcium 40, 73 (Jul, 2006).
4. K. V. Kuchibhotla, et al., Science 323, 1211 (Feb 27, 2009).
5. K. V. Kuchibhotla et al., Neuron 59, 214 (Jul 31, 2008).

Disclosures: Y. Yang: None. **H. Uhlirova:** None. **S.L. Gratiy:** None. **P.A. Saisan:** None. **L. Reznichenko:** None. **E.S. Norheim:** None. **Q. Cheng:** None. **K.L. Weldy:** None. **S. Sakadžić:** None. **J.M. Conner:** None. **J. Cui:** None. **T. González:** None. **D.A. Boas:** None. **G.T. Einevoll:** None. **A.M. Dale:** None. **E. Masliah:** None. **A. Devor:** None.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.04/M5

Topic: C.04. Parkinson’s Disease

Support: Univ. Bordeaux Segalen

CNRS

Fondation de France (grant 16810)

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Title: Synaptic transmission at cortico-subthalamic synapses

Authors: L. FROUX[1], S. MORIN[1], B. BIOULAC[1], J. BAUFRETON[1], J. LANCIEGO[2], *A. I. TAUPIGNON[1];
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Abstract: The subthalamic nucleus (STN) is widely viewed as a relay nucleus in basal ganglia (bg). However, motor and premotor cortical inputs to STN have been demonstrated. In addition, cortical inputs arising from associative and limbic areas have recently been described, suggesting that STN integrates contextual information to set a decisional threshold (1). The cortical inputs to the STN form the so-called ‘hyperdirect pathway’ of bg.

Dopamine receptors in D1 and D2 families have been identified on afferent terminals and at post-synaptic sites within STN (2). The cortico-subthalamic (Cx-STN) transmission may therefore be modulated by dopamine and changes in dopamine at the synapses may contribute to the symptoms of Parkinson's disease and other neuropsychiatric disorders.
The data on the hyperdirect pathway and the hypothesis on its role(s) call for determining the properties of the Cx-STN synapses and clarifying the action of dopamine.
Contrary to the direct and indirect pathways of bg, the hyperdirect pathway is usually not preserved in in vitro preparations. Besides, bulk stimulations of afferent cortical fibers in the internal capsule may mask properties specific of the various cortical projections. To circumvent these two problems, we used optogenetics to specifically stimulate corticofugal axons in acute brain slices. We stereotactically delivered a viral expression vector in the motor cortex of mice in vivo, and at least 14 days later, made acute brain slices. We mostly found YFP-expressing cell bodies in cortex layer V and YFP-expressing fibers in various nuclei of bg and in the ventro-medial part of STN. We used patch clamp to probe the effects of photo-activating ChR2. A single laser blue light flash directed at YFP-expressing fibres in the STN evoked robust inward currents with graded amplitude properties in line with the duration and power of photostimuli. Average peak current amplitude and charge, were -144.41 ± 31.71 pA and -544.83 ± 93.24 pA.ms (n=7), respectively, at -80 mV. The mean delay from flash to current onset was 1.91 ± 0.11 ms (n=7), a value consistent with synaptic delay. The currents were consistently abolished by TTX (0.5 μM) or by DNQX (20 μM). Thus, light activated ChR2 which in turn elicited action potentials in ChR2-expressing pre-synaptic cortical fibres/terminals, induced glutamate release and ultimately produced AMPA/kainate EPSCs in post-synaptic STN neurons.
These results support the view that optogenetics is a valuable method to determine the properties of the motor Cx-STN pathway.
1. W. I. Haynes, S. N. Haber, J. Neuroscience 33, 4804 (Mar 13, 2013)
2. K. S. Rommelfanger, T. Wichmann, Front. in Neuroanat. 4, 139 (2010)

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Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

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Program#/Poster#: 240.05/M6

Topic: C.04. Parkinson's Disease

Support: Envoy Therapeutics

Michael J Fox Foundation for Parkinson Research

NS 064439

Title: L-DOPA-induced dyskinesia is associated with enhanced H2 histamine excitation of striatal cholinergic interneurons

Authors: *S. A. LIM, R. XIA, L. WON, Y. DING, D. S. MCGEHEE, U. J. KANG;
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Abstract: Levodopa is the most effective therapy for Parkinson's disease (PD), but long term treatment leads to the development of potentially debilitating abnormal involuntary movements, or L-DOPA-induced dyskinesia (LID). Amantadine is the most effective treatment for LID at present, but its effectiveness is limited by a short therapeutic time window and worsening of LID symptoms following cessation of treatment. We recently demonstrated that striatal cholinergic interneuron (ChI) dysfunction is associated with mouse models of LID. ChI physiological changes include enhanced baseline excitability and enhanced dopamine-induced excitation. There is strong histaminergic innervation of the dorsal striatum and excitatory histamine receptors are expressed on ChIs, which lead us to test the contribution of this system to LID-induced elevation of striatal cholinergic tone. Two mouse models of PD were used, the unilateral 6-hydroxy-dopamine (6-OHDA) lesion and the Pitx3ak/ak aphakia mouse. Stepping tests were performed to determine success of the 6-OHDA lesion and the onset of Parkinsonian-like symptoms. Mice from each group were chronically treated with either vehicle or L-DOPA to induce LID. The cylinder test was performed to quantify the severity of dyskinesia. Brain slice electrophysiology was used to assess the histamine sensitivity of ChIs from dyskinetic mice as well as control mice. In cell-attached extracellular recordings, we found that H2 receptor-mediated excitation of striatal ChIs was enhanced in mice expressing LID. In the behaving animal, H2 receptor blockade by systemic administration of famotidine decreased LID expression. Similar effects of H2 antagonists were seen in both models of PD. Our data suggest that LID is associated with enhanced H2-mediated histamine signaling, and that inhibiting H2 receptors may decrease the LID-induced hypercholinergic state of the striatum. Histamine H2 receptors may therefore be a novel target for alleviating LID symptoms in humans.

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Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.06/M7

Topic: C.04. Parkinson's Disease

Support: Supported by grant 152326 from Conacyt México.

Title: Dopamine D3 receptor prevents D1 receptor stimulation of [3H]GABA release in substantia nigra pars reticulata of hemiparkinsonian dyskinetic rats

Authors: *S. ALBARRAN[1], A. ÁVALOS-FUENTES[2], F. PAZ-BERMÚDEZ[1], D. ERLIJ[3], J. ACEVES[1], B. FLORÁN*[1];
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Abstract: L-DOPA remains as the most effective replacement therapy for Parkinson disease. Long term L-DOPA treatment leads to the development of dyskinesia. The mechanisms involve in dyskinesia remains unclear, but dopamine D1 receptors play a central role. Dopamine D1 and D3 receptors are coexpresed in striato-nigral neurons were dopamine D3 receptors potentiate D1 receptor stimulation of cAMP formation and GABA release. L-DOPA treatment increases the expression of D3 receptor correlating with the development of L-DOPA priming. This data originate the proposal of use dopamine D3 receptor agents as a therapeutic target for attenuation of dyskinesia but, results are still contradictory. Since GABA release in the substantia nigra reticulata (SNr) is determinant for the develop of dyskinesia, we study the effect of dopamine D3 receptor activation on D1 receptor stimulated [3H]GABA release and [3H]cAMP formation in slices and synaptosomes of SNr in the unilateral 6-OHDA lesioned rat model treated with L-DOPA divided in dyskinetic and non-dyskinetic groups. In non-dyskinetic rats, dopamine D3 receptor activation potentiates GABA release and cAMP formation stimulated by D1 receptor agonist SKF 38393. The release of GABA was similar to that observed in the control animals. In the dyskinetic rats the D3 receptor activation inhibits D1 receptor stimulation of GABA release and cAMP formation suggesting an antagonist interaction between these receptors. The total amount of GABA released by D1 receptor in this condition was higher than in the normal side. This data suggested an antagonistic interaction between D1 and D3 receptor in dyskinesia and suggested the use of agonist for the D3 receptor in the management of L-DOPA induce dyskinesia.

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Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.07/M8

Topic: C.04. Parkinson's Disease

Support: NINDS K08 NS069625

NCCAM T32 AT002688

Title: Alpha-synuclein aggregation begins at presynaptic terminals

Authors: *K. SPINELLI, J. TAYLOR, V. OSTERBERG, C. MESHUL, V. UNNI;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Parkinson's Disease and Dementia with Lewy Bodies are associated with abnormal aggregation of the synaptic protein alpha-synuclein. However, the mechanisms governing aggregation in vivo are poorly understood. We have developed an in vivo multiphoton imaging paradigm to study the process of alpha-synuclein aggregation in living mouse cortex with subcellular resolution. Using in vivo multiphoton fluorescence recovery after photobleaching (FRAP), we demonstrate that somatic alpha-synuclein exists as an unbound, soluble protein. In contrast, alpha-synuclein in presynaptic terminals exists in at least 3 different pools. One pool is unbound, soluble protein, while another is bound to presynaptic vesicles, and the final pool consists of microaggregate species. Chronic imaging of terminal microaggregates over 1 week demonstrates a heterogeneous population, with likely differing levels of compaction. Using a combination of biochemical techniques, we confirm the presence of a terminal aggregate species that is resistant to Proteinase K digestion, phosphorylated at serine-129 and oxidized, and is separate from the vesicle-bound fraction by EM. In addition, we have used multiphoton FRAP to measure, for the first time in vivo, the specific binding constants for alpha-synuclein's binding to synaptic vesicles and its effective diffusion coefficient in the soma and axon. Collectively, this paradigm introduces powerful new tools for studying protein aggregation in living mouse brain, and suggests that under moderate overexpression conditions mimicking human disease, alpha-synuclein aggregation begins first at presynaptic terminals in vivo. Ongoing experiments investigate the ability of curcumin, a natural anti-aggregate found in turmeric spice, to decrease aggregation of alpha-synuclein in vivo.

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Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

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Program#/Poster#: 240.08/M9

Topic: C.04. Parkinson's Disease

Support: Grant-in-Aid for Scientific Research (B) JSPS KAKENHI (24390345)

Title: Effect of subthalamic stimulation on nonmotor symptoms of advanced Parkinson disease

Authors: *Y. MIYAGI[1,2], T. OKAMOTO[2], K. SAMURA[3], Y. OHYAGI[2], H. HONDA[2]; [1]Dpt.Stereotactic and Functional Neurosurg., Kaizuka Hosp., Fukuoka, Japan; [2]Kyushu Univ., Fukuoka, Japan; [3]Dpt. Neurosurg., Fukuoka Univ., Fukuoka, Japan

Abstract: Most patients with advanced Parkinson disease (PD) have nonmotor symptom (NMS). NMSs are not life-threatening but considerably affect their quality of life, therefore the management of NMS is one of the top agendas in PD. Chronic subthalamic stimulation (STN-DBS) improves the "off"-related motor symptoms but its effect on "off"-related NMS (nonmotor fluctuation, NMF) is still unclear. We have developed an online questionnaire of NMS of PD on the basis of the report by Witjas et al. (2002), which included 54 symptoms classified into categories; 26 dysautonomic, 21 psychiatric/cognitive and 7 sensory symptoms. The presence, timing ("on"- or "off"-related, "on/off"-unrelated) and severity (0, absent; 1, slightly uneasy; 2, moderately uneasy; 3, severely uneasy; 4, intolerable) of each symptom were collected from 34 PD patients before and after 6 months of STN-DBS. Among NMFs ("off"-related NMS), the top 10 rankings of decreased prevalence (prevalence before DBS minus that after DBS) of NMF were; sadness 33%, slowness of thinking 30%, fatigue 24%, urinary urgency 21%, irritability 21%, abulia 21%, sensation of injustice 18%, dyspnea 18%, self-withdrawal 18%. All severities of these NMFs and akathisia were significantly alleviated after STN-DBS ($p<0.05$, Wilcoxon paired signed rank test). The alleviated NMFs included 8 psychiatric, 2 dysautonomic and 1 sensory symptoms. Among "on/off"-unrelated NMSs, 4 NMSs were significantly alleviated after STN; mental hyperactivity, constipation, hallucination and flatulence. STN-DBS alleviates not only "off"-related NMS but also some of "on/off"-unrelated NMSs probably through the improvement of motor function and/or the decrease of dopamine replacement therapy.

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Poster

240. **Parkinson's Disease: Circuits and Cells**

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Topic: C.04. Parkinson's Disease

Support: NIH Grant NS041071

Title: Aging exacerbates motor deficits in a parkinsonian mouse model

Authors: *T. A. ANSAH, T. NAYYAR;
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Abstract: Parkinson's disease (PD) is characterized by degeneration of nigrostriatal dopamine (DA) neurons and a decrease in striatal DA concentration. The decrease in striatal DA levels is thought to underlie the motor symptoms of PD. Majority of PD cases is idiopathic, affecting 1 - 2% of persons over the age of 65 years. Data suggesting that striatal DA concentrations decline as a function of age has led to the hypothesis that PD arises from the effects of some acute event, such as exposure to a neurotoxicant, superimposed on the age-related decline in striatal dopamine, ultimately reaching a threshold for manifestation of symptoms. Evidence in support of this hypothesis comes from studies showing that the environmental neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces more severe nigrostriatal dopamine neuron degeneration in older than young animals. Assessment of motor function in MPTP-treated mice has yielded inconsistent results. Some of the variance across reports probably reflects differences in age at the time of MPTP treatment and the survival time leading up to behavioral testing. In the current studies we investigated whether aging will exacerbate motor deficits produced by MPTP treatment. Mice were treated with MPTP or saline and striatal DA and motor function were assessed three weeks or 18 months after MPTP treatment. MPTP treatment resulted in 80% reduction in striatal DA at three weeks survival time. At 18 months after MPTP treatment the striatal DA was found to be 50% of that observed in saline treated mice. MPTP treatment had no effect on locomotor activity as well as performance on the rotarod apparatus when measured three weeks after MPTP treatment. In contrast significant impairments in motor function were observed 18 months after MPTP treatment. The data suggest that aging plays a significant role in interacting with environmental toxicants to produce parkinsonian motor symptoms.

Disclosures: T.A. Ansah: None. **T. Nayyar:** None.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.10/M11

Topic: C.04. Parkinson's Disease

Title: L-DOPA-induced dyskinesia involve structures outside the basal ganglia

Authors: *M. BASTIDE, G. CHARRON, S. DOVERO, C. E. GROSS, P.-O. FERNAGUT, E. BEZARD;
Gironde, Instute of Neurodegenerative Dis., BORDEAUX, France

Abstract: The most effective symptomatic therapy for Parkinson's disease (PD) remains the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA). Long-term treatment leads to involuntary aimless movements called L-DOPA-induced dyskinesia (LID). The stimulation of the dopamine D1 receptor pathway results in increased expression of several molecular markers, in particular the members of the immediate-early gene (IEG) family, a class of genes rapidly transcripted in response to an external stimulus. As the primary target of nigral DA neurons, the striatum has received most attention to understand the pathophysiology of LID. However, the myriad of dopaminoceptive structures in the brain that are likely to be affected by the exogenously produced DA have received little, if any, attention although they might play a key role in mediating those L-dopa-induced abnormal behaviours.
Therefore, we here characterized ΔFosB, ARC, Fra2 and Zif/268 IEGs expression in the whole brain of dyskinetic and non-dyskinetic rats to identify brain nuclei displaying a transcriptional response specifically related to LID and not to the treatment itself. For this mapping, we quantified the number of positive IEGs-cells using unbiased stereological methods. Within the basal ganglia, the striatum and the substantia nigra reticulata showed an increased expression for all the IEGs between the dyskinetic and non-dyskinetic rats on the lesioned side. Outside the basal ganglia, there was an increased expression of the 4 IEGs in the Motor Cortex, 3 nuclei of the bed nucleus of the stria terminalis, the hippocampus, the pontine nuclei and the cuneiform nucleus. Moreover, the Zona incerta and the lateral habenula displayed an overexpression of ΔFosB, ARC and Zif268. These results illustrate a global transcriptional response to a dyskinetic state in the whole brain suggesting the possible involvement of these structures in LID.

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Poster

240. **Parkinson's Disease: Circuits and Cells**

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Topic: C.04. Parkinson's Disease

Support: NIH grant NS 59910

Title: Nicotinic receptor drugs reduce L-dopa-induced dyskinesias in a nonhuman primate model of Parkinson's disease

Authors: *M. QUIK[1], D. ZHANG[1], D. SOHN[1], F. I. CARROLL[2], M. DECKER[3];
[1]Ctr. for Hlth. Sci., SRI Intl., Menlo Park, CA; [2]RTI Intl., Research Triangle Park, NC; [3]AbbVie, Inc., North Chicago, IL

Abstract: L-dopa is one of the primary therapies for Parkinson's disease. However, its long term use results in the development of abnormal involuntary movements or dyskinesias for which there are currently few treatment options. Accumulating data now indicate that nicotine treatment results in a decline in L-dopa-induced dyskinesias in several parkinsonian animal models, suggesting that this may represent a useful treatment approach. However, nicotine may act at multiple sites yielding beneficial results but also side effects. The goal of the current study was to determine whether selective nicotinic receptor drugs reduce L-dopa-induced dyskinesias in parkinsonian nonhuman primates, a model that resembles Parkinson's disease. Monkeys were given MPTP over several months until parkinsonian. They were then gavaged with L-dopa (10 mg/kg) plus carbidopa (2.5 mg/kg) twice daily, with dyskinesias scored every 30 min throughout the course of the day. We first examined the effect of varenicline, a drug that interacts with multiple nicotinic receptors, to test the hypothesis that the effect of nicotine is receptor-mediated. Varenicline (provided by F.I.C.) reduced L-dopa-induced dyskinesias in a dose dependent manner by ~50%. ABT-089 (provided by AbbVie, Inc.), an agonist that interacts selectively with β2* nicotinic receptors, also decreased L-dopa-induced dyskinesias by ~50%. This compares well to the effect of nicotine which reduced dyskinesia by 55%. The effect of these drugs persisted for the entire length of the study (several months). Parkinsonism was not modified with drug treatment. These data suggest that β2* nicotinic receptor subtype drugs may be useful for the treatment of L-dopa-induced dyskinesias in Parkinson's disease patients. Importantly, varenicline has been approved for use in humans as a smoking cessation aid and ABT-089 has been evaluated in phase 2 clinical trials for other indications. These drugs could thus rapidly be repurposed and approved for clinical studies to test their ability to reduce L-dopa-induced dyskinesias in Parkinson's disease.

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Poster

240. **Parkinson's Disease: Circuits and Cells**

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Daniel F. and Ada L. Rice Foundation

The Center for Compulsive Behavior and Addiction

Title: Rats that self-administer methamphetamine show Parkinson's disease-like inflammation and α-synuclein pathology in the brain and colon

Authors: *T. NAPIER, S. M. KOUSIK, L. P. KELLY, S. M. GRAVES, A. L. PERSONS; Rush Univ. Med. Ctr., CHICAGO, IL

Abstract: Methamphetamine (meth) is a potent psychostimulant, and abuse of meth is associated with an increased risk for developing Parkinson's disease (PD) (Callaghan *et al. Drug Alcohol Depend* 120:35, 2012). Underlying mechanisms may include oxidative stress, mitochondrial dysfunction and inflammation (Yamamoto *et al. Ann. NY Acad. Sci.* 1187:101,2010), factors that are also believed to be involved in PD pathology and disease progression. Studies of meth-induced toxicity are largely based on *in vivo* experiments using acute, high doses of non-contingently administered meth. It is unknown if similar outcomes occur with protocols that better emulate human drug-taking, e.g., self-administration of more "physiologically relevant" doses of meth. The current study determined if meth self-administration increased markers of inflammation i.e., glial fibrillary acidic protein (GFAP; a marker of glial cell activation) in the rostral striatum and substantia nigra, the terminal and cell body regions (respectively) of dopaminergic projections that are most severely affected in PD. To validate the concept that meth abuse enhances vulnerability for PD, we then assayed the substantia nigra for α-synuclein, a neuropathological hallmark for the disease. Accumulating evidence in the PD field suggests that peripheral factors may contribute to central degenerative processes; specifically, α-synuclein aggregates have been identified in the enteric nervous system of the colon of PD patients prior to the onset of motor symptoms (Shannon *et al. Mov Disord.* 27:709,2012). Thus, we also assayed the gut for α-synuclein and GFAP. Male Sprague-Dawley rats were trained to self-administer meth for 14 days; controls were saline-yoked. Tissue was harvested on day 15 and prepared for Western blotting (brain) or immunohistochemistry (colon). Meth self-administration increased GFAP expression in the rostral striatum and nigra ($p<0.01$). In the nigra, α-synuclein levels were also increased ($p<0.01$). Quantitative assessments of α-synuclein and GFAP optical density in the colon revealed increased staining in meth-treated rats compared to controls ($p<0.05$). Collectively, these results provide the first evidence for PD-like pathology in the brains and colon of meth self-administering rats, and therefore offer insights into mechanisms that underlie the enhanced vulnerability of meth abusers to develop PD later in life. It is of great interest to

ascertain if detection of α-synuclein in the colon can serve as a biomarker that predicts PD in meth abusers.

Disclosures: T. Napier: None. **S.M. Kousik:** None. **L.P. Kelly:** None. **S.M. Graves:** None. **A.L. Persons:** None.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.13/M14

Topic: C.04. Parkinson's Disease

Support: Kenneth Douglas Foundation

Daniel F and Ada L Rice Foundation

Center for Compulsive Behavior and Addiction, Rush Univeristy

Title: Methamphetamine self-administration reduces dopamine in the nigrostriatal and mesolimbic pathways in association with ischemia suggesting methamphetamine abuse as a risk factor for Parkinson's disease

Authors: *S. M. KOUSIK[1], T. C. NAPIER[1,2], P. M. CARVEY[1];
[1]Pharmacol., [2]Psychiatry, Rush Univ. Med. Ctr., Chicago, IL

Abstract: Methamphetamine (meth) causes excess dopamine (DA) release and can be neurotoxic. Meth abuse may be a risk factor for Parkinson's disease (PD); an alarming event as worldwide meth abuse is about 26 million. Meth abusers display reduced DAergic function and some motor deficits similar to PD patients. Laboratory animal models suggest these DA losses result from increased DA-quinones, reactive oxygen species and/or hyperthermia, but were based on extremely high levels of meth that would not likely be self-administered by humans. We focused on patterns of DA neurotoxicity in male SD rats following meth self-administration for 14 days (rats self-titrated in the 3hr/day sessions to about 4mg/kg/day) followed by forced abstinence for 1, 14, 28, or 56 days. Saline-yoked rats served as controls. Tyrosine hydroxylase (TH) was used to identify DA terminals and neurons. In the dorsal striatum, there was a 17% TH reduction ($p<0.05$) at 14 days withdrawal, and a 50% reduction ($p < 0.001$) at 28 and 56 days. TH+ cells stereologically counted in the substantia nigra, the bed nucleus of striatal terminals, revealed a 22% loss ($p < 0.05$) of TH+ cells at both 28 and 56 days withdrawal. To verify that TH reductions represented actual DA terminal and cell loss and not suppression of the TH

phenotype, a retrograde tracer (Fluorogold (FG)) was injected into the striatum and evaluated in the nigra to indicate active connections. FG imaging revealed a reduction in FG transport to the nigra at 28 and 56 days withdrawal suggesting actual terminal and cell loss within the nigrostriatal pathway. These DA losses were associated with significant vasoconstriction of small-diameter vessels (0.01-0.03 mm) in the dorsal striatum at 1 and 14 days but not 28 and 56 days (assessed using micro-computed tomography) suggesting dysfunction of the neurovascular unit. TH was also reduced (50%; $p<0.001$) in the nucleus accumbens (NAc)-core at 28 and 56 days withdrawal, but not the NAc-shell. Accordingly, FG transport from the lateral ventral tegmental area to the NAc-core was reduced at 56 days withdrawal. In summary, meth self-administration resulted in early dysfunction within the nigrostriatal sensorimotor pathway, similar to PD pathology. This was followed by dysfunction within the NAc-core, i.e., the "limbic-motor interface." The NAc-shell, which mediates reward-seeking behavior, maintained normal DA function. These data reveal a meth-induced dysfunction in the neurovascular unit producing initial ischemia which results in actual DA losses consistent with PD pathology.

Disclosures: S.M. Kousik: None. **T.C. Napier:** None. **P.M. Carvey:** None.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.14/M15

Topic: C.04. Parkinson's Disease

Support: NIH Grant R01-NS059600

Title: The anti-dyskinetic effects of serotonin transporter blockade are partially reversed by 5-HT_{1A} receptor antagonism

Authors: *M. CONTI, P. SINGH, C. BISHOP;
Psychology, Binghamton Univ., Binghamton, NY

Abstract: Dopamine (DA) replacement therapy with L-DOPA is standard treatment for Parkinson's disease (PD), but chronic treatment usually leads to the development of abnormal involuntary movements (AIMs) referred to as L-DOPA-induced dyskinesia (LID). Accumulating work has shown that compensatory plasticity in serotonin (5-HT) neurons contributes to LID leading researchers to directly target serotonin autoreceptors to attenuate LID. Treatment with 5-HT_{1A} agonists inhibits L-DOPA-derived DA release from 5-HT neurons thus alleviating LID but chronic treatment may increase motor disability. Recent evidence has indicated that 5-HT transporter (SERT) blockade with selective 5-HT reuptake inhibitors (SSRIs) also provides anti-

dyskinetic protection without interfering with motor performance. A possible mechanism underlying anti-dyskinetic effects of SSRIs may be indirect activation of 5-HT_{1A} receptors. The current investigation sought to test this mechanism using unilateral 6-OHDA medial forebrain bundle lesions in Sprague-Dawley rats. After a 3 week recovery period, rats were primed with L-DOPA (6 mg/kg + benserazide 15 mg/kg; s.c.) and monitored for AIMs expression. Using a within subjects design, rats received the following treatment across 10 test days spaced 3-4 days apart: vehicle or the 5-HT_{1A} antagonist WAY100635 (0.5 mg/kg) was administered 5 min prior to vehicle or SSRI treatment with either citalopram (3 or 5 mg/kg) or paroxetine (0.5 or 1.25 mg/kg) which was administered 30 min prior to L-DOPA. Rats were tested for ALO AIMs for 3 h immediately following L-DOPA treatment. Behavioral results indicated that blocking 5-HT_{1A} receptors partially reversed SSRIs attenuation of LID. Findings indicate that SSRIs benefits as co-treatment with L-DOPA are in part mediated by indirect stimulation of 5-HT_{1A} receptors.

Disclosures: M. Conti: None. **P. Singh:** None. **C. Bishop:** None.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.15/M16

Topic: C.04. Parkinson’s Disease

Support: Intramural Research Program, NIDA

Title: L-dopa induced dyskinesias in Parkinsonian mice

Authors: *B. J. HOFFER[1], L. OLSON[2], C. BACKMAN[3];
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[3]Intramural Res. Program, NIDA, Baltimore, MD

Abstract: In Parkinson's disease (PD), the efficacy of L-dopa treatment is gradually lost over time, as dyskinesias emerge in response to previously beneficial doses of the drug. Using MitoPark mice, a transgenic model that closely mimics the gradual loss of dopamine (DA) neurons observed in PD, we found that the level of DA denervation and associated adaptations in striatal neurotransmission at the time of L-dopa treatment determines the development and escalation of L-dopa-induced dyskinesias. MitoPark mice show a slow progressive loss of DA afferents in limbic areas starting in adulthood, in which nigrostriatal dopaminergic projections innervating the dorsal striatum are strongly affected, while mesolimbic projections innervating the ventral striatum are relatively spared, a pattern that closely mimics the progression of PD in humans. In this study we chronically treated 20-week old (moderate DA loss), and 28-week old

(severe DA loss) Mitopark mice with L-dopa (10 mg/kg i.p. twice a day). The locomotor response after the first and subsequent L-dopa treatments differed significantly between 20-week and 28-week old MitoPark mice. While all MitoPark mice developed locomotor sensitization to L-dopa treatment over time, 28-week old mice showed a decreased latency and increased response duration to the first and subsequent L-dopa injections. In parallel with this data, MitoPark mice with a high degree of DA striatal denervation (28-week old) developed abnormal involuntary movements (AIMs) rapidly and severely after starting L-dopa treatment, as compared to the more gradual escalation of AIMs in animals that started treatment at earlier states of the degenerative process (20-week old). Our data suggests that it is the loss of DA innervation and subsequent adaptations in the striatum that may set up the tone in the limbic system for how motor complications develop with L-dopa treatment. PCR array studies of striatal neurotransmitter receptors revealed profound changes in the mRNA expression levels for various DA, serotonin, glutamate and GABA receptors in the striatum of 30-week old Mitopark mice. While, chronic L-dopa treatment starting at 20 weeks of age normalized the expression pattern of some neurotransmitter receptors, it exacerbated, or in cases inverted, the expression of others. This study highlights the relevance of this newly developed mouse model as an enhanced platform that offers clinically relevant similarities to the manifestation of L-dopa induced motor complications during PD, and therefore could be utilized in the preclinical development of new pharmacological and gene therapies aimed at correcting the manifestation of motor complications in PD.

Disclosures: B.J. Hoffer: None. **L. Olson:** None. **C. Backman:** None.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.16/M17

Topic: C.04. Parkinson’s Disease

Support: CIHR MOP-119347

MJFF The Role of LRRK2 in Neurotransmission

LRRK2 in neuronal architecture and membrane dynamics

Title: The interplay between LRRK2 and the striatal DA system influences behavior in genetic animal models

Authors: *M. VOLTA[1], D. A. BECCANO-KELLY[1], L. N. MUNSIE[1], P. CHOU[1], S. BERGERON[1], S. CATALDI[1], I. TATARNIKOV[1], K. CO[1], L. TAPIA[1], H. MELROSE[3], A. J. MILNERWOOD[2], M. J. FARRER[1];
[1]Med. Genet., [2]Med., Univ. of British Columbia, Vancouver, BC, Canada; [3]Neurosci., Mayo Clin., Jacksonville, FL

Abstract: Genetic variability in leucine-rich repeat kinase 2 (LRRK2) is a major risk factor for sporadic and familial Parkinson's disease (PD). In LRRK2 transgenic mice neuronal microarchitecture is perturbed and several studies have reported age-associated dysfunction in nigrostriatal dopamine (DA) signalling reminiscent of late-stage PD; Yue and Lachenmayer, 2011). Nevertheless, early albeit subtle deficits in neuronal physiology, and associated behavioural correlates, warrant further investigation. Herein we report a detailed behavioural characterization of several LRRK2 lines (vs. non-transgenic littermates) focused on motor and non-motor phenotypes relevant to the symptomatology of patients with PD. We observe DA-dependent behavioural alterations at 3-6 months in LRRK2 knockout (KO) and BAC hWT-LRRK2 mice supported by aberrant striatal DA signalling. Based on these observations we have broadened our study to include a recently developed LRRK2 G2019S knock-in (KI) line. In these animals expression of endogenous albeit mutant LRRK2 protein facilitates physiologic investigation of the effects of the pathogenic mutation. Initial data in heterozygous KI animals point to a trend for increased spontaneous locomotor activity in the open field at young age-points (3 and 6 months). In parallel, we report on subtle cognitive abilities (puzzle box test), spatial orientation and stereotypies (Y-maze test). To investigate the interplay between LRRK2 and the DA system across different models, KI mice have been challenged with DA receptor ligands and tested in the open field. At the same time, the expression levels of DA-related proteins has been assessed in the striata, including quantitative analyses of DN neurone (SNc) number, striatal axon terminal density, DA receptor and DA-related signalling proteins and their localization. Based on preliminary findings we hypothesize that early, altered DA signalling in striatal cells (rather than nigral neuronal death) heralds the initial pathophysiology of LRRK2-parkinsonism. The data suggest that LRRK2-parkinsonism is a developmental disorder that necessitates compensatory synaptic and circuitry changes for successful aging. Our result provides a comparative report of LRRK2's in vivo role, revealing several neuronal mechanism(s) underlying PD-relevant behavioural phenotypes. Ultimately, this data and these animals may inform therapeutic efforts aimed at disease modification (neuroprotection), to slow or halt disease progression.

Disclosures: M. Volta: None. **D.A. Beccano-Kelly:** None. **L.N. Munsie:** None. **A.J. Milnerwood:** None. **I. Tatarnikov:** None. **P. Chou:** None. **S. Bergeron:** None. **S. Cataldi:** None. **K. Co:** None. **L. Tapia:** None. **H. Melrose:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Foundation. **M.J. Farrer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Foundation.

F. Consulting Fees (e.g., advisory boards); International Editorial board for Parkinsonism and Related DisordersIn, Parkinson Society of Canada Scientific Advisory Board member, Michael J. Fox Foundation Executive Scientific Advisory Board Member, EURAC Scientific Advisory Council member.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.17/M18

Topic: C.04. Parkinson's Disease

Support: CIHR MOP-119347

MJFF Role of Lrrk2 in neuronal transmission

MJFF Lrrk2 in neuronal architechture and membrane dynamics

Title: Early synaptic alterations in LRRK2 G2019S Knock-In mice

Authors: *D. A. BECCANO-KELLY[1], M. VOLTA[1], L. MUNSIE[1], I. TATARNIKOV[1], P. CHOU[1], K. CO[1], S. BERGERON[1], L. TAPIA[1], H. L. MELROSE[4], L. A. RAYMOND[2], A. J. MILNERWOOD[3], M. J. FARRER[1];
[1]Med. Genet., [2]Psychiatry, [3]Neurol., Univ. of British Columbia, Vancouver, BC, Canada; [4]Neurosci., Mayo Clin., Jacksonville, FL

Abstract: Parkinson's disease (PD) is the most prevalent neurodegenerative movement disorder in the world, affecting ~2% of the population. Current therapeutic research focuses on late-stage clinical phenotypes which, at best, only alleviate symptoms rather than prevent the disease progression. In order to design preventative therapies it is crucial to determine early pathophysiological changes in PD models.
Mutations in the gene encoding the Leucine-Rich Repeat Kinase 2 (LRRK2) protein cause the highest morbidity in familial and idiopathic PD cases, with the G2019S mutation alone accounting for ~2% of PD cases.
The neuronal function of LRRK2 is still unknown, although recent research has implicated it in glutamate release in simple model systems and dopamine transmission in the striatum of aged transgenic mice. Many current investigations use BAC mice overexpressing (OE) human LRRK2 to draw conclusions about PD; however results are difficult to interpret due to higher expression levels, species-irrelevant expression patterns and random insertion events.
In this study we characterize early synaptic alterations in Knock-In (KI) mice carrying the

G2019S mutation in the LRRK2 murine homologue with endogenous control of expression. Whole-cell electrophysiological recordings revealed alterations in glutamatergic transmission onto striatal medium spiny neurones in acute brain slices from 1 month old KI mice. Spontaneous excitatory postsynaptic currents display increased frequency and decreased amplitudes furthermore, this effect is related to allelic copy number with homozygous mice having a more pronounced effect than heterozygous mice.
Preliminary data also suggest altered dopamine transmission at this age in KI mice as assayed by fast scan cyclic voltammetry.
Western blotting further revealed altered levels of numerous striatal receptors and signal transduction proteins pertinent to dopamine transmission including LRRK2 itself. The data suggest a consequence of the G2019S mutation is altered LRRK2 auto-regulation.
The data show that a single point mutation, mimicking a PD risk factor, results in early synaptic phenotypes in mice in a manner distinct from those seen in either OE or knock-out mice. Thus G2019S KI mice reveal differences in LRRK2 physiology induced by mutations that are neither simplistic loss nor gain of function. We demonstrate that early stage alterations precede late stage deficits and provide a target for preventative therapeutics against onset and progression of PD.

Disclosures: D.A. Beccano-Kelly: None. **M. Volta:** None. **L. Munsie:** None. **I. Tatarnikov:** None. **P. Chou:** None. **K. Co:** None. **S. Bergeron:** None. **L. Tapia:** None. **H.L. Melrose:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Foundation. **L.A. Raymond:** None. **A.J. Milnerwood:** None. **M.J. Farrer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Foundation. F. Consulting Fees (e.g., advisory boards); International Editorial board for Parkinsonism and Related Disorders, Parkinson Society of Canada Scientific Advisory Board member, Michael J. Fox Foundation Executive Scientific Advisory Board member, EURAC Scientific Advisory Council member.

Poster

240. **Parkinson's Disease: Circuits and Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 240.18/N1

Topic: C.04. Parkinson’s Disease

Support: STINT Foundation

Swedish Research Council

EU-FP7

Title: Morphological adaptations of 'direct pathway' spiny projection neurons in experimental parkinsonism and L-DOPA-induced dyskinesia

Authors: T. FIEBLINGER[1], C. ALCACER[1], L. ZANETTI[1], S. M. GRAVES[2], J. L. PLOTKIN[2], D. J. SURMEIER[2], *M. A. CENCI[1];
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Abstract: The dendrites of striatal spiny projection neurons (SPN) are the principal anatomical site where dopaminergic and glutamatergic inputs to the striatum functionally interact to control movement (Gerfen and Surmeier, Annu. Rev. Neurosci. 34: 441-66, 2011). Previous studies have shown that SPN forming the 'indirect pathway' (iSPN) undergo dendritic spine pruning and loss of corticostriatal synapses in rodent models of Parkinson´s disease (PD). In this study, we set out to examine the dendritic adaptations of 'direct pathway' SPN (dSPN), which play a pivotal role in the development of motor complications during the treatment of PD with L-DOPA (Cenci, Trends Neurosci 30:236-243, 2007). BAC transgenic mice expressing TdTomato under the control of the D1 receptor promoter sustained unilateral 6-OHDA lesions of the medial forebrain bundle followed by a 10-day treatment with either L-DOPA or vehicle (saline). L-DOPA was given at dose inducing severe dyskinesia (6-12 mg/kg/day i.p.; Francardo et al. Neurobiol. Dis. 42:327-40, 2011). TdTomato-positive neurons were patched and filled with Alexa-488 in corticostriatal slices. Dendritic morphology and spine numbers were examined in optical z-stacks acquired with a two-photon laser microscope. Following 6-OHDA lesions, dSPN showed a significantly reduced total dendritic length, fewer branch points, and lower numbers of Sholl intersections ($p < 0.05$ vs normal controls on all parameters). This dendritic regression was accompanied by a significant increase in intrinsic excitability, as determined from current-voltage plots. Subsequent dyskinesiogenic treatment with L-DOPA did not significantly modify the dendritic regression of dSPN, nor their intrinsic excitability. However, it produced an approximately 30% reduction in spine density ($p < 0.05$ vs both controls and 6-OHDA-saline treated mice). These results show that dSPN undergo dendritic regression following dopamine denervation. This regression is neither aggravated nor alleviated by a short course of dyskinesiogenic treatment with L-DOPA. The treatment however results in a significant loss of spines. Ongoing studies are examining the possible significance of these dendritic adaptations to the pathophysiology of L-DOPA-induced dyskinesia.

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Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 241.01/N2

Topic: C.04. Parkinson's Disease

Support: AASDAP

FINEP

INCT - INCEMAQ

FAPERN

CAPES

The Swedish Research Council [#325-2011-6441], Crafoord Foundation

The Michael J Fox Foundation Target Validation Fall 2010 Grant

Title: Characterization of long-term motor deficits in the 6-OHDA model of Parkinson's disease in the common marmoset

Authors: *M. SANTANA[1,2], T. PALMER[3,4], H. SIMPLÍCIO[5,6,7], P. PETERSSON[3], M. NICOLELIS[5,8,9,10,11], R. FUENTES[5];
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Abstract: Research aimed at developing new therapies for Parkinson's disease (PD) critically depend on valid animal models of the disease that allows for repeated testing of motor disabilities over extended time periods. We here present a through characterization of a wide range of motor symptoms in the 6-OHDA marmoset model of PD when tested over several months. Severity of motor deficits were quantified both through manual scoring protocols appropriately adapted to include species specific motor behavior, through the use of automated quantitative motion tracking procedures based on mathematical image analysis of digital video

recordings and recordings by actimetry sensor. Analyses show that the automated methods allow for rapid and reliable characterization of changes in motor behavior matching the manual scoring procedures and that robust motor symptoms lasting for several months could be induced when using a two-stage neurotoxic lesioning procedure involving one hemisphere at a time (Mitchell et al. 1995). This non-human primate model of PD may therefore be well suited for long-term evaluation of novel therapies for treatment of PD.

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Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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CAPES

Title: Distribution and morphology of calcium-binding neurons following chronic multielectrode implants

Authors: *M. A. FREIRE[1,2], E. MORYA[2], J. FABER[1,3,4], J. R. SANTOS[1,5], N. A. M. LEMOS[1], M. A. L. NICOLELIS[2,6,7,8];
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Abstract: The development of therapies to improve the quality of life of people suffering from different types of body paralysis through the reestablishment of its sensory and motor functions

is a major medical challenge. Brain-machine interface (BMI) emerges as a potential answer, by allowing the control of paralyzed body parts directly by brain activity. The chronic implant of multielectrodes, employed to record neural activity directly from the brain parenchyma, is a fundamental component of an invasive BMI. However, before this technique can be effectively available to human clinical trials, it is necessary to comprehensively characterize its impact on the nervous tissue. In the present work we evaluated the impact of chronic implanted tungsten multielectrode arrays on the distribution and morphology of calcium-binding protein (CBP) neurons (CB), calretinin (CR), and parvalbumin (PV) across rat's motor cortex. In addition, to estimate the impact of post-implant elapsed time on neuronal recordings, we also correlated the cell distribution with their electrophysiological signals, which were assessed by comparing the total number of neuronal units observed in the first and last recording sessions, in order to evaluate their signal-to-noise ratio (SNR). Our results point out that chronic electrode arrays were well tolerated by the nervous tissue, with recordings remaining viable for up to 6 months after implantation, presenting a peak of signal quality around 3 months of implant. In addition, both morphology and distribution of CBP neurons were not broadly affected. Moreover, both a restricted neuronal loss and glial activation were observed on the implanted sites. Altogether, our results suggest that tungsten multielectrodes can be deemed as a feasible candidate to future human clinical interventions involving BMI.

Disclosures: M.A. Freire: None. **E. Morya:** None. **J. Faber:** None. **J.R. Santos:** None. **N.A.M. Lemos:** None. **M.A.L. Nicolelis:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Support: Michael J Fox Foundation

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Parkinson Research Foundation

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AASDAP

Title: Effects of spinal cord stimulation on the neuronal population dynamic of the cortico-basal ganglia-thalamic circuit in a primate model of Parkinson´s disease

Authors: *P. HALJE[1], M. SANTANA[2,3], H. SIMPLÍCIO[2,4,5], M. NICOLELIS[2,6], R. FUENTES[2], P. PETERSSON[1];
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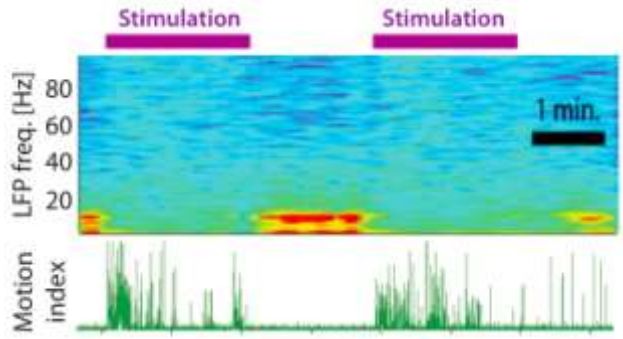
Abstract: Spinal cord stimulation (SCS) has been proposed as a novel therapy for symptomatic treatment of Parkinson's disease (PD) based on rodent experiments and a few clinical case reports. We here show that dorsal SCS at the thoracic level reduce PD symptoms in unilaterally 6-OHDA lesioned marmosets and normalize neural activity in some - but not all - structures in the cortex-basal ganglia system.
The animals (n=2) were implanted with chronic epidural stimulation electrodes with the anode and cathode on different sides of the midline. We also implanted chronic 64-channel microwire electrodes positioned bilaterally in motor cortex, somatosensory cortex, putamen, subthalamic nucleus and the ventral lateral and ventral posterolateral thalamic nuclei.
The analyses of the local field potentials (LFPs) in the different structures showed that activity specific to the PD state, such as cortical over-expression of beta oscillations, was effectively normalized by the SCS. A comparison of the activity during SCS with the activity during levodopa treatment revealed several similarities but also a few remarkable differences in the spectral profile of the LFPs in the different structures; motor cortex, putamen and subthalamic nucleus showed similarities, while somatosensory cortex and thalamus (ventral lateral and ventral posterolateral nuclei) showed differences compared to the levodopa treated state.
Also, we evaluated the effect of different stimulation frequencies on behavior and electrophysiology. Tested stimulation frequencies ranged from 4 Hz to 300 Hz and behavioral effects were quantified offline using video tracking software. We found that the optimal stimulation frequency varied greatly between individuals. In motor cortex the resemblance of the spectral profile to the levodopa treated state correlated with symptom relief. In contrast, the spectral profile in putamen primarily showed a higher degree of resemblance to the levodopa state as stimulation frequency increased, but did not directly correlate to reduction of symptoms



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Disclosures: P. Halje: None. **M. Santana:** None. **H. Simplício:** None. **M. Nicolelis:** None. **R. Fuentes:** None. **P. Petersson:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 241.04/N5

Topic: C.04. Parkinson’s Disease

Support: Michael J Fox Foundation

Swedish Research Council 325-2011-6441

Olle Engkvist Foundation

Åke Wiberg Foundation

Parkinson Research Foundation

Crafoord Foundation

Title: Functional coupling in the cortico-basal ganglia-thalamic loop in a primate model of Parkinson's disease

Authors: *U. RICHTER[1,2], P. HALJE[2,3], M. SANTANA[4,5], M. NICOLELIS[4,6,7,8,9], R. FUENTES[4], P. PETERSSON[2,3];
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Abstract: The cerebral cortex, the basal ganglia, and thalamus together constitute an anatomically distributed but strongly interconnected neuronal network that is known to play a crucial role for motor behavior. A deeper knowledge of the relevance of functional coupling within this neural network is of crucial interest for a better understanding of both normal and pathophysiological behavior, in particular in Parkinson's disease.
We have here studied functional coupling in terms of coherence between the motor cortex (MI), somatosensory cortex (SI3a), putamen (Put), subthalamic nucleus (STN) and the ventral lateral (VL) and ventral posterolateral (VPL) thalamic nuclei of unilaterally 6-OHDA lesioned marmosets (n = 2). The animals were bilaterally implanted with chronic 64-channel microwire electrodes, and neuronal recordings were obtained in the freely moving animals during a Parkinsonian baseline period and following therapeutic levodopa treatment. Time-resolved coherence was calculated for all available pairs of local field potential signals. Video recordings were employed to quantify the overall motor activity, which was correlated to the mean coherence in frequency bands of interest.
In subject 01 an intermittent narrowband oscillation in the 10-Hz band could be observed in the Parkinsonian state in several structures of the lesioned, but also in a number of channels of the intact hemisphere. The mean coherence around this oscillation frequency (8-12 Hz) was significantly increased in the Parkinsonian compared to the levodopa-treated state. Furthermore, in the Parkinsonian state the strength of the coherence was significantly modulated with motor activity, such that it decreased at the occasions when the animal displayed relatively normal motor activity.
In subject 02 a more broadband intermittent oscillation in the 10 to 20-Hz band was observed in the Parkinsonian state, where it was most prominent in MI and Put of the lesioned hemisphere. Similar to subject 01 the mean coherence between MI and Put was stronger in the lesioned compared to the intact hemisphere and was negatively correlated to motor activity. A similar trend could also been seen between MI and VL as well as Put and VL of the lesioned hemisphere, and levodopa treatment effectively reduced coherence between all structures.
These findings are in line with a pathophysiological role of low-frequency oscillations causing excessive synchronization and functional coupling between MI, Put, STN and VL in the cortico-basal ganglia-thalamic loop, thereby potentially preventing functional information processing and resulting in motor disturbances.

Disclosures: U. Richter: None. **P. Halje:** None. **M. Santana:** None. **M. Nicolelis:** None. **R. Fuentes:** None. **P. Petersson:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

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Program#/Poster#: 241.05/N6

Topic: C.04. Parkinson's Disease

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Michael J Fox Foundation for Parkinson's Research (Target Validation Program Fall 2010)

FINEP

INCT Incemaq CNPq n. 610009/2009-5

FAPERN

CAPES

The Swedish Research Council [#325-2011-6441], Crafoord Foundation

Title: Effects of spinal cord stimulation on the neuronal firing rate and synchronization of the cortico-basal ganglia-thalamic circuit in a primate model of Parkinson´s disease

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Abstract: Epidural electrical stimulation of the spinal cord (SCS) for chronic pain dramatically improves tremor, rigidity, posture and gait in Parkinson´s disease (PD), as reported in 18 patients from four different clinical studies (Parkinsonism Relat Disord. 2012; 18:213-4; Neurol Med Chir (Tokyo). 2012, 52(7):470-4; Neuromodulation 2012 Dec 10; J Clin Neurosci. 2013 pii: S0967-5868(13). Nonetheless, the neural mechanisms underlying these effects are largely unknown. We hypothesize that SCS alleviates the motor symptoms by modulating the activity of supraspinal structures related to sensory and motor function.
Objective: To study the effect of spinal cord stimulation in the neuronal activity of the cortico-basal ganglia-thalamic circuit in a primate model of PD.
Methods: Adult males marmosets (Callithrix Jacchus) received unilateral or bilateral medial forebrain bundle 6-OHDA lesions and were later chronically implanted with a spinal cord bipolar stimulation electrode at high thoracic level, EMG wires in the deltoid muscles, and two 32-channel microelectrode arrays bilaterally targeting the 6 following structures: primary motor cortex, somatosensory cortex, putamen, ventral lateral and ventral posterior lateral thalamus, and

subthalamic nucleus. Electrophysiological and video recordings were obtained from marmosets with different levels of dopamine depletion while freely moving in an open field.
Results: SCS caused alleviation of parkinsonian symptoms. Analysis of the firing rate showed that SCS consistently modulated the neuronal firing in all the structures recorded, mainly in the ventral lateral thalamus, ventral posterior lateral thalamus, and subthalamic nucleus. Additionally, in the parkinsonian condition, nearly one third of the recorded neurons showed oscillatory activity in the beta range, which was reduced while SCS was on.
Conclusion: The firing rate modulation and the disengagement of neuronal activity from pathological beta rhythm in most of the supraspinal structures recorded may represent important physiological changes induced by SCS that lead to alleviation of motor symptoms.

Disclosures: R.A. Fuentes: None. **P. Petersson:** None. **M. Santana:** None. **H. Simplicio:** None. **T. Palmer:** None. **M.A.L. Nicolelis:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

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Program#/Poster#: 241.06/N7

Topic: C.04. Parkinson’s Disease

Support: Michael J Fox Foundation (2010 Target Validation)

AASDAP, FINEP, INCT Incemaq, FAPERN, CAPES

The Swedish Research Council [#325-2011-6441], Olle Engkvist Foundation, Åke Wiberg Foundation, Parkinson Research Foundation and Crafoord Foundation.

Title: A method for characterization of activity patterns in cortico-basal ganglia-thalamic structures related to reaching in a primate model of Parkinson’s disease

Authors: *P. PETERSSON[1,2], P. HALJE[1], M. SANTANA[2], R. FUENTES[2], M. A. L. NICOLELIS[2,3,4,5,6];
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Abstract: During behavioral tests, 6-OHDA lesioned marmosets commonly show difficulties in controlling skilled limb movements and may sometime display motor freezing at the moment of movement initiation when trying to reach for food rewards.

In order to further characterize these types of motor deficits in this primate model of Parkinson's disease and to investigate the associated neurophysiological processes in the cortico-basal ganglia-thalamic loop, we have here characterized reaching behavior in two unilaterally 6-OHDA lesioned adult male marmoset monkeys that were trained in a skilled reaching task requiring the use of both forelimbs (adapted from Campos-Romo et al. 2009).
Animals were trained over a period of several weeks to reach and grasp small pieces of marshmallow through holes in a vertical wall. When the task was fully learnt the animals would normally collect all food rewards within less than 5 min. Both animals were exposed to neurotoxic lesions affecting the medial forebrain bundle according to previously described protocols (adapted from Annette et al. 1992) and eight weeks later, were implanted with bilateral 64-channel wire electrode arrays targeting primary motor cortex and parts of somatosensory cortex, putamen, the subthalamic nucleus and the ventral lateral and ventral posterolateral thalamic nuclei in both hemispheres. Two weeks after implantation, the animals were tested in the reaching set-up and it was confirmed that the animals had retained the ability to perform the task.
Preliminary analyses of the recorded data from these two subjects indicate that parallel single unit recordings could be obtained from the different structures of the cortico-basal ganglia-thalamic loop in all recording sessions during the performance of the task and that freezing events could easily be identified from the digital video recordings.
By analyzing neuronal unit activity specifically related to periods of behavioral freezing as well as other detailed aspects of normal reaching behavior a more comprehensive understanding of neuronal dynamics in the cortico-basal ganglia-thalamic circuit related to voluntary skilled motor control of the hand will be obtainable.

Disclosures: P. Petersson: None. **P. Halje:** None. **M. Santana:** None. **R. Fuentes:** None. **M.A.L. Nicolelis:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Program#/Poster#: 241.07/N8

Topic: C.04. Parkinson's Disease

Support: Action Plan Brazil- Switzerland CNPq 590006/2010-0

FINEP

INCT

Incemaq

AASDAP

FAPERN

CAPES

Title: Chronic Spinal Cord Stimulation alleviates motor asymmetry in an Alpha-synuclein animal model of Parkinson Disease

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Abstract: Parkinson Disease (PD) is associated with progressive loss of dopaminergic neurons in the nigrostriatal pathway and aberrant low-frequency synchronous corticostriatal oscillations. Electrical Spinal Cord Stimulation (SCS) is a minimal invasive neuromodulation method that showed to be effective in alleviating parkinsonian motor symptoms in 6-hydroxydopamine lesioned rats, dopamine depleted mice and PD patients. The mechanisms underlying the pro-kinetic effects of SCS remain poorly understood. Alpha-synuclein overexpression provides a progressive model of dopaminergic destruction, suitable for both testing potential therapies and study the underlying mechanisms.
Objective: To conduct a behavioral, electrophysiological and immunohistochemical characterization of the AAV6 alfa-synuclein rat model of PD and to test the chronic effects of SCS in this model.
Experiment 1) 2 µl of empty vector suspension AAV6 (Control) or of the vector carrying the gene for wild type human alpha-synuclein (Unil, Unil+SCS Groups) were injected stereotaxically in the right substantia nigra of Sprague-Dawley male rats. Animals were tested for asymmetry in the Cylinder test one week before and weekly from 4th to 10th week after injection. SCS groups were submitted to stimulation sessions once a week from 6th to 10th week in an Open Field.
Experiment 2) Electrode arrays were implanted in both hemispheres in motor cortex and dorsolateral striatum of rats injected with vector suspension AAV6 alpha-synuclein in the right substantia nigra and empty viral suspension in the left. Electrophysiological recording was performed during the behavioral tests from 1th to 10th week following the viral injection. Brains were collected and submitted to basic histology and immunohistochemistry.
Results: There is no difference between groups in the distance walked during the Open Field Test ($p>0.05$). Animals from Unil and Unil+SCS groups showed a significant decrease in the use

of the forepaw contralateral to the lesion (left) four weeks after viral injection in the Cylinder Test [$F(3,23)=5.2$, $p<0.05$]. However, animals treated with chronic SCS (Unil+SCS) recovered the ability to use the left paw after the SCS sessions. In the 6th week after the viral injection the mean percentage of left forepaw use in the Unil Group was 15.17%, while in the SCS Group was 39.04%.
Conclusion: Chronic SCS alleviates motor asymmetry in an animal model of PD based on the overexpression of the protein alpha-synuclein in the substantia nigra. Chronic epidural stimulation showed to be an effective treatment to motor PD symptoms and might become an alternative neuromodulation option to treat PD.

Disclosures: I. Brys: None. **B. Schneider:** None. **M. M. Freire:** None. **M. Nicolelis:** None. **R. Fuentes:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Program#/Poster#: 241.08/N9

Topic: C.04. Parkinson's Disease

Support: AASDAP

FINEP

INCT Incemaq

FAPERN

CAPES

Action Plan Brazil-Switzerland CNPq 590006/2010-0

Title: Expression efficiency of rAAV6 and rAAV9 serotypes in the ventral midbrain of common marmosets

Authors: *T. FAGGION VINHOLO[1,2], M. B. SANTANA[1,6], M. F. P. ARAÚJO[1], H. SIMPLÍCIO[1,7,8], B. SCHNEIDER[9], R. FUENTES[1], M. A. L. NICOLELIS[1,3,4,2,5], M. A. M. FREIRE[1];
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Natal, Brazil; [7]Fac. of Hlth. Sci., State Univ. of Rio Grande do Norte, Mossoró, Brazil; [8]Neurosurgical, Neuron, Natal, Brazil; [9]École Polytechnique Federale de Lausanne, Lausanne, Switzerland

Abstract: The development of animal models through protein expression by viral infection is an attractive approach for neuropathology studies. The recombinant adeno-associated viral (rAAV) vectors provide a novel tool to study the neurodegeneration observed in Parkinson's disease (PD). Different rAAV serotypes differ in their expression efficiency. In the current study, we compared the expression in the primate ventral midbrain of two different viral strains, AAV6 and AAV9, using as model common marmosets (Callithrix jacchus). Both vectors carried Green Fluorescent Protein (GFP) as a marker for viral efficacy. The injections were conducted in three marmosets weighing 300g-400g according to a modified protocol from Brain 2007. 130:799-815. Briefly, 2 injections of 2µL of virus per hemisphere were performed, both targeting ventral midbrain. The animals received an injection of the AAV6 strain on the right hemisphere and one of AAV9 strain in the left hemisphere. Before and after the lesions, the animals were evaluated using a rating scale of motor impairment adapted from UDPRS. After 8 months the animals were perfused (0.9% warm heparinized saline solution and 4% cold paraformaldehyde) and the brain tissues were processed for histological analyses. Representative slides of the putamen and substantia nigra were stained with GFP immunohistochemistry. Subsequently, images were captured in a confocal microscope (Zeiss LSM 710, 40x objective) and cell count was done in the substantia nigra images. After the injection the animals did not present any behavioral change, asserting that GFP can be used as a control marker protein in the marmoset brain. There was a difference of GFP expression between the hemispheres, indicating a difference in the viral efficiency and suggesting that AAV9 is more efficient than AAV6 for viral injection. Accordingly, AAV9 might be a better prospective to be used as a viral carrier for a disease model in future studies, such as studies with PD models in the common marmoset.

Disclosures: T. Faggion Vinholo: None. **M.B. Santana:** None. **M.F.P. Araújo:** None. **H. Simplício:** None. **B. Schneider:** None. **R. Fuentes:** None. **M.A.L. Nicolelis:** None. **M.A.M. Freire:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Program#/Poster#: 241.09/N10

Topic: C.04. Parkinson's Disease

Support: AASDAP

FINEP

INCT incemaq

FAPERN

CAPES

Action Plan Brazil-Switzerland CNPq 590006/2010-0

Title: Analysis of local field potentials in a common marmoset (Callithrix jacchus) during rest and locomotion

Authors: *M. F. P. ARAUJO[1], R. C. MOIOLI[1], F. L. BRASIL[1], R. FUENTES[1], M. A. L. NICOLELIS[1,2,3,4,5];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder in which the most striking symptoms are related to motor impairments. Traditionally, rodents are used as animal models to study the mechanisms and possible treatments of PD. Recently, common marmosets (Callithrix jacchus) have been increasingly used in PD research. So far, however, the neurophysiological processes related to locomotor behavior in this primate species are still not well established. The aim of this work was to investigate the neurophysiological processes at the cortico-basal ganglia-thalamic loop related to rest and spontaneous locomotion periods.
To examine locomotor network activity we implanted 64 microeletrodes bilaterally to record neuronal spike and local field potentials (LFP) in 4 regions of a female marmoset monkey: primary motor cortex, putamen, internal globus pallidus and external globus pallidus. At each recording session, the monkey was placed inside a plexiglass box (40 cm x 40 cm), where she was allowed to freely move for 10 min. The monkey behavior was recorded by 2 video-cameras: top and frontal. LFPs during movement and rest period were successfully recorded from the 4 structures in all recording sessions.
Preliminary analyses indicate that LFPs successfully relate to locomotor acitivity in all video analyses in this species. Together, this experimental setup and the resulting data will provide a better understanding of the functioning of the cortico-basal ganglia-thalamic circuit of common marmosets during rest and spontaneous locomotion periods. In addition, these results provide the grounds for the analysis of this circuit in different marmoset models of PD.

Disclosures: M.F.P. Araujo: None. **R.C. Moioli:** None. **F.L. Brasil:** None. **R. Fuentes:** None. **M.A.L. Nicolelis:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Program#/Poster#: 241.10/N11

Topic: C.04. Parkinson's Disease

Support: NINDS NS078100

University of Iowa Aging Mind & Brain Initiative

Title: Prefrontal dopamine is essential for temporal control

Authors: *K. L. PARKER, N. S. NARAYANAN;
Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Parkinson's disease is a devastating neurodegenerative disorder that impairs movements. However, PD patients also suffer significant morbidity from cognitive symptoms of this disease. Temporal control of movement, or how movements are guided in time to achieve behavioral goals, is one such cognitive deficit. One contributor to these symptoms may be dysfunctional dopamine signaling in cognitive brain regions such as the prefrontal cortex. We have previously shown that disrupting prefrontal D1 dopamine signaling impairs interval timing and conversely, optogenetic stimulation facilitates temporal control. Here, we record from prefrontal neuronal ensembles in awake, behaving rats performing an interval timing task while pharmacologically manipulating dopamine signaling within these brain regions. We trained rats to perform an interval timing task with a 12 second interval. Once well trained, an injection cannula and electrode arrays were implanted in the prelimbic cortex. Prelimbic neuronal activity and local field-potentials were analyzed during interval timing performance, and while D1 antagonists (SCH 23990) and D1 agonists (SKF 81297) were infused. We found three major patterns of prefrontal neuronal activity: 1) timing-encoded ramping activity, 2) reward-acquisition-related activity, and 3) motor-related activity. Following D1 blockade, animals' interval-timing performance was impaired. Furthermore, ramping activity among prefrontal neurons was less prominent in sessions with D1 blockade, while neuronal activity related to movement and reward-acquisition was preserved. Finally, time-frequency analysis revealed that task-modulated low-frequency rhythms were altered. Taken together, these data suggest that D1 dopaminergic systems within prefrontal cortex are critical to temporal control, and suggest that manipulating D1 systems may be a useful target of novel pharmacological agents that treat cognitive symptoms of Parkinson's disease.

Disclosures: K.L. Parker: None. **N.S. Narayanan:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson's Disease

Support: NINDS K08 NS078100

UIowa AMBI

Title: Dorsal raphe serotonergic neurons and levodopa-induced dyskinesia in Parkinson's disease

Authors: *V. CERPA, A. D. MILLER, G. B. RICHERSON, N. S. NARAYANAN;
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder involving degeneration of midbrain dopaminergic neurons. L-3,4-dihydroxyphenylalanine (Levodopa; L-Dopa) is an effective treatment for the motor symptoms of PD; however, L-Dopa therapy commonly leads to disabling side effects including motor fluctuations and dyskinesias. L-Dopa does not induce dyskinesias in humans without PD. In primate models of PD, dyskinesias require dopaminergic denervation. Prior work has demonstrated that dyskinesias can be modulated by 5-HT1a/5-HT1b receptor agonists; however, it is unclear which serotonin neurons, projections, and striatal circuits are involved. Serotonin nuclei do degenerate in PD, although much less than monoaminergic nuclei. Here we use tract tracing, in-vivo microdialysis, optogenetics, and electrophysiology to map the influence of serotonin signaling on L-Dopa-induced dyskinesias in a mouse 6-OHDA model of PD. First, using retrograde tracers in Pet1-YFP mice, we identified a population of neurons in the dorsal raphe that directly project to the dorsal striatum. We found that both dorsal raphe serotonin and non-serotonin neurons monosynaptically project to the striatum. Next, we found that dyskinesia severity correlated with dopaminergic damage, and dyskinesias were diminished after co-administration of L-Dopa and administration of the 5-HT1a receptor agonist 8-OH-DPAT. We used in vivo microdialysis to analyze striatal neurochemistry during L-Dopa-induced dyskinesias and administration of 5-HT1a receptor agonists. Future directions will explore L-Dopa-induced dyskinesias with optogenetic stimulation of serotonin neurons in the dorsal raphe, as well as with striatal electrophysiology. Our results describe a direct projection between serotonin projections from the dorsal raphe nucleus to the striatum. This projection could be targeted by future therapies aimed at reducing L-Dopa-induced dyskinesias.

Disclosures: V. Cerpa: None. **A.D. Miller:** None. **G.B. Richerson:** None. **N.S. Narayanan:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Program#/Poster#: 241.12/N13

Topic: C.04. Parkinson's Disease

Support: R01NS70872

Title: Subthalamic nucleus deep brain stimulation induces differential functional activation of the motor, associative, and basal ganglia circuit in a nonhuman primate

Authors: *P. H. MIN[1], S. HAN[3], E. ROSS[1], M. MARSH[1], J. JEONG[4], S.-Y. CHANG[1], C. D. BLAHA[5], K. BENNET[2], K. H. LEE[1];
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Abstract: Deep brain stimulation (DBS) within the basal ganglia complex is an effective neurosurgical approach for treating a variety of neurological and psychiatric disorders, including Parkinson's disease (PD). Despite well-established clinical efficacy, the mechanism of DBS remains poorly understood. To help elucidate the mechanism of action of DBS, we performed 3T functional magnetic resonance imaging (fMRI) in a Rhesus monkey. We stereotactically implanted a miniature 6 contact DBS electrode into the subthalamic nucleus (STN), passing through zona incerta, dorsal STN, medial STN, ventral STN and to substantia nigra. We tested the proportional effects of each contact DBS by manipulating current spread and varying stimulation contacts in a within-subjects design. The fMRI examinations were performed using a simple block-style paradigm that alternated between the stimulator-off condition and the stimulator-on condition using DBS parameters (1-6V, 0.15 msec pulse width at 130 Hz) similar to those used clinically. High frequency stimulation of the STN in anesthetized monkey induced local blood oxygenation level dependent (BOLD) effect in the ipsilateral motor cortex, ipsilateral caudate, and contralateral cerebellum. Importantly, stimulation of both STN and SN resulted in intensity-dependent activations of the BOLD signal. Our results demonstrate that DBS activates areas of the brain, distal to the electrodes, suggesting that DBS activates a diffuse neural network that may be involved in alleviating motor dysfunction in PD patients. Taken together, our results

show that the nonhuman primate model for DBS fMRI, which conforms to human implanted DBS electrode configurations and human neuroanatomy, may be a useful platform for translational studies investigating the global neuromodulatory effects of DBS.

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Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson’s Disease

Support: Rosalind Franklin University of Medicine and Science

The PRI innovation grant

The Parkinson's Disease Foundation

Title: Dopamine depletion results in altered frequency dependent striatal responses to cortical stimulation

Authors: *V. R. JAYASINGHE[1], D. R. THOMASES[2], A. R. WEST[3], K. Y. TSENG[2]; [2]Cell. and Mol. Pharmacol., [3]Neurosci., [1]Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Parkinson’s disease is a common neurodegenerative disorder. It is characterized by the loss of dopamine (DA) neurons in the substantia nigra that project primarily to the striatum. Despite the well-defined motor deficits in Parkinson’s disease, the neurobiological mechanisms underlying such impairments are not clear. Recent studies revealed that the emergence of the parkinsonian state is often associated with an increased synchronous activity at beta frequencies (15-30 Hz) between the basal ganglia and cortex. These excessive oscillations are thought to disrupt cortico-striatal transmission and thereby result in the motor symptoms characteristic of Parkinson’s disease. Here we employed *in vivo* local field potential recordings to determine if striatal processing of synchronous cortical inputs at high frequencies is impaired following chronic DA depletion. Towards this goal, we used the 6-hydroxydopamine rat model of parkinsonism and assessed the pattern of striatal response to motor cortex stimulation using trains of pulses delivered at 10, 20, or 40 Hz. In control/DA-intact animals (sham-lesioned rats), we found that motor cortex stimulation at 10 and 20 Hz typically elicited a marked inhibition

local field potential response in the dorsal striatum. Interestingly, such suppression of striatal local field potential responses is lacking in the DA-depleted group. In contrast, striatal response to 40 Hz stimulation appears to remain intact following DA depletion. Thus, an intact DA system within the striatum is required for the normal suppression of cortical inputs at 10 and 20 Hz, but not at 40 Hz. Together, these results are indicative of an impaired inhibitory control of cortical inputs in the DA-depleted striatum, and suggest that the augmented cortico-basal ganglia synchronization observed in the parkinsonian state is not restricted to beta oscillations.

Disclosures: V.R. Jayasinghe: None. **D.R. Thomases:** None. **A.R. West:** None. **K.Y. Tseng:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 241.14/N15

Topic: C.04. Parkinson’s Disease

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Title: Characterization of four stages of neural changes in parkinsonism using cross-frequency-coupling analysis of subthalamic nucleus local field potentials

Authors: *T. H. SANDERS[1], A. DEVERGNAS[2], M. A. CLEMENTS[1], T. WICHMANN[2,3]; [1]Georgia Inst. of Technol., Atlanta, GA; [2]Udall Ctr. of Excellence in PD Res., Atlanta, GA; [3]Dept. of Neurology, Emory Univ., Atlanta, GA

Abstract: The progression of Parkinson's disease (PD) can be modeled in primates with repeated small injections of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Much has been learned about PD using this model. However, we have insufficient knowledge regarding the link between dopamine loss, altered brain activities and parkinsonian motor signs. To address this issue, we analyzed subthalamic nucleus (STN) local field potentials (LFPs) recorded in monkeys before, during, and after induction of parkinsonism with MPTP injections.

The analyzed data were recorded in two rhesus monkeys rendered parkinsonian with weekly i.m.

injections of MPTP. STN-LFPs were recorded with bipolar recording methods as the monkeys progressed from baseline to moderate parkinsonism. An observational rating scale was used to quantify the level of parkinsonism.
We used phase-amplitude cross frequency coupling (CFC) features extracted from the STN LFP data for the analysis. We found that, while beta power was not changed consistently in these animals, ANOVA showed in both monkeys statistically significant increased beta phase modulation compared to baseline conditions for slight to mild parkinsonism, followed by decreased beta phase modulation for the moderate parkinsonism conditions. Analysis of the relationship between parkinsonism and the relative magnitude of 36 phase-amplitude CFC features (delta through gamma bands) over increasing severity of motor signs revealed several discernible stages of parkinsonism, each with a characteristic pattern of phase-amplitude modulation. The progression for each monkey was statistically similar. The data suggest that worsening parkinsonism is associated with predictable patterns of brain activity changes.

Disclosures: T.H. Sanders: None. **A. Devergnas:** None. **M.A. Clements:** None. **T. Wichmann:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson’s Disease

Support: NIH 1R21NS078660

NIH 1R01NS081716

NSF- DMS-1042134

Title: Causal role of striatal cholinergic system in generating parkinsonian beta oscillations

Authors: *X. GU[1], M. MCCARTHY[2], N. KOPELL[2], X. HAN[2];
[1]Biomed. Engin., [2]Boston Univ., Boston, MA

Abstract: Enhanced beta oscillations (15-30Hz) are found in the cortical-basal ganglion-thalamic neural circuit of parkinsonian brains and correlate with motor dysfunction in Parkinson’s disease. However, it remains unclear how the loss of dopaminergic neurons in the substantia nigra in Parkinson’s disease leads to pathologically enhanced beta oscillations. Dopamine neurons in the substantia nigra primarily project to the striatum, and among their

broad effects on various striatal neuronal types, they inhibit striatal cholinergic interneurons. Our computational models have suggested that elevated cholinergic tone can turn a normal striatum into a beta oscillation pacemaker, primarily through elevating the firing rate of medium spiny neurons in the indirect pathway. Direct infusion of the cholinergic agonist carbachol into the striatum of normal mice elicited profound beta oscillations, suggesting that normal striatum contains sufficient neural elements to generate beta oscillations and that acetylcholine is a potent modulator of striatal beta rhythms. To further examine the causal role of the striatal cholinergic system in generating beta oscillations in Parkinson's disease, we used optogenetics to directly modulate striatal cholinergic interneurons. Direct optogenetic activation of striatal cholinergic neurons lead to enhanced beta oscillations similar to that induced with striatal carbachol infusion but with much more precise temporal control over their onset and offset. In addition, optogenetic silencing of striatal cholinergic neurons in 6-hydroxydopamine parkinsonian mice models restored parkinsonian motor deficits. Together, our results provide direct evidence for the causal role of cholinergic interneurons in generating parkinsonian beta oscillations and related motor deficits.

Disclosures: X. Gu: None. **M. McCarthy:** None. **N. Kopell:** None. **X. Han:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson's Disease

Support: NIH CRCNS Grant R01 NS46058

NSF-DMS-1042134

Title: Deep brain stimulation control of beta oscillations through the indirect pathway: A computational study

Authors: *M. M. MCCARTHY, N. KOPELL;
Boston Univ., BOSTON, MA

Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) can be highly effective in alleviating movement disability in Parkinson's disease. However, its therapeutic mechanism of action is unknown. DBS is most effective at reducing motor pathology if the stimulation frequency is high (~130 Hz). Beta frequency DBS is either ineffective or may worsen motor symptomatology. These findings suggest that interruption of beta rhythms in basal

ganglia networks may be instrumental in the therapeutic action of DBS. Previously we have shown that robust beta oscillations can be generated in the striatum of normal mice under a condition relevant to Parkinson's disease: high cholinergic tone. Computational modeling suggests that robust beta rhythms can emerge in networks of striatal medium spiny neurons (MSNs) in the presence of high cholinergic tone due to increased interaction between the synaptic GABAa current and the intrinsic membrane M-current. Here we extend our models to study how DBS may be working to interfere with the production of exaggerated beta rhythms in striatal networks. We find that a subset of neurons in the external segment of the globus pallidus (GPe) which projects to the striatal MSNs is capable of normalizing the MSN network when driven to spike at high frequency (~ 130 Hz). Specifically, with high frequency input from this subset of GPe neurons, the parkinsonian MSN network returns to a normal baseline spiking rate and the beta power within the network returns to normal levels. Conversely, we find that input to the striatum from the GPe neurons at beta frequency increases the beta power in MSN networks. We show that beta frequency GABAergic input to the MSN network creates resonance within the network, increasing beta frequency spiking of the population of MSNs, whereas high frequency (130 Hz) input to the MSN network creates a condition similar to static inhibition of the network, which opposes the increased excitation due to high cholinergic tone. These findings suggest the therapeutic mechanism of DBS to the STN may work through network dynamics in the indirect pathway of the basal ganglia.

Disclosures: M.M. McCarthy: None. **N. Kopell:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson's Disease

Support: European Comission grant Neuron-48-013

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European Comission grant FP7-PEOPLE-2009-IEF-253965

Title: A direct relationship between parkinsonian rest tremor and oscillatory coupling in a distributed subcortico-cortical network

Authors: *J. HIRSCHMANN[1], C. J. HARTMANN[1], M. BUTZ[2], N. HOOGENBOOM[1], T. E. ÖZKURT[3], S. ELBEN[1], J. VESPER[1], L. WOJTECKI[1], A. SCHNITZLER[1];

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Abstract: Electrophysiological studies on parkinsonian rest tremor revealed that neuronal oscillations and muscle activity are synchronized at tremor frequency, suggesting that oscillatory coupling might underlie tremor manifestation. However, it has not been demonstrated so far that the strength of oscillatory coupling is indeed related to the presence and severity of tremor. To shed light on the relationship between tremor and coupling, we took advantage of naturally occurring spontaneous tremor fluctuations and measured oscillatory coupling both during the presence and absence of rest tremor.
We simultaneously recorded local field potentials (LFPs) from the subthalamic nucleus (STN), the magnetoencephalogram (MEG) and the EMG of forearm muscles in 11 patients with Parkinson's disease. Recordings took place the day after surgery for deep brain stimulation, following withdrawal of anti-parkinsonian medication. We selected epochs containing spontaneous rest tremor and tremor-free epochs, respectively, and compared power and coherence between STN, cortex and muscle across conditions. Tremor-induced increases in coherence were localized by Dynamic Imaging of Coherent Sources (Gross et al., PNAS 2001). Subsequently, cortico-cortical coupling was investigated by reconstruction of the time domain activity of selected sources and computation of the imaginary part of coherency, a coupling measure insensitive to volume conduction (Nolte et al., Clin. Neurophysiol. 2004).
Following tremor onset, LFP power decreased in the beta band (13-30 Hz) and increased at double the individual tremor frequency. Sensor level STN-cortical, cortico-muscular and STN-muscular coherence increased during tremor specifically at individual tremor frequency. The increase in STN-cortical coherence correlated with the increase in EMG power. On the source level, we observed tremor-induced increases in cortico-muscular coherence in primary motor cortex, premotor cortex and posterior parietal cortex contralateral to the tremulous limb. Analysis of the imaginary part of coherency revealed tremor-dependent coupling between these cortical areas at individual tremor frequency and double tremor frequency.
This study demonstrates that the STN is a part of a distributed subcortico-cortical tremor network (Timmermann et al., Brain 2003). Furthermore, it reveals that spontaneous tremor manifestation is associated with characteristic changes in STN power and intra-network oscillatory coupling.

Disclosures: J. Hirschmann: None. **C.J. Hartmann:** None. **M. Butz:** None. **N. Hoogenboom:** None. **T.E. Özkurt:** None. **S. Elben:** None. **J. Vesper:** None. **L. Wojtecki:** None. **A. Schnitzler:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson's Disease

Support: FP7 Neuwalk

Title: Translational analysis platform for neuromotor disease research and therapeutic validation: Application to Parkinson's Disease

Authors: I. VOLLENWEIDER[1], Y. LANG[2], D. BORTON[1], *D. W. KO[3], Q. LI[3], G. COURTINE[1], E. BEZARD[2];
[1]Ctr. for Neuroprosthetics and Brain Mind Inst., Swiss Federal Inst. of Technol. (EPFL), Lausane, Switzerland; [2]Inst. of Neurodegenerative Dis., Bordeaux, France; [3]Motac Neurosci. Ltd, Greater Manchester, United Kingdom

Abstract: Parkinson's disease (PD) patients experience progressive motor symptoms that become associated with medication-induced motor complications, including motor fluctuations and dyskinesia. The past 2 decades have seen unprecedented preclinical and clinical efforts towards identifying and validating putative targets to establish novel antiparkinsonian therapies. While a multiplicity of therapeutic interventions has been developed in rodent models, only a few were tested in MPTP-treated monkeys, which remain the gold standard for modelling PD in non-human primates. The lack of consensus on a methodology, and the almost exclusive reliance upon the sole clinical rating of PD symptoms in the MPTP-treated monkeys, have been among the major roadblocks that have hindered successful translation to clinical settings. Consequently there is a critical need to develop high-resolution translational methodologies to evaluate safety and optimize efficacy of therapeutic interventions developed in rodents before clinical applications. Here, we leveraged our newly established translational analysis platform to characterize alteration of whole-body kinematic, muscle activity, and motor cortex neuronal modulations during unconstrained locomotion in MPTP-treated monkeys. The animals were trained to perform a range of natural walking tasks designed to evaluate the contribution of specific neural pathways to gait control. Using principal component analysis applied on a large number of multifaceted parameters, we could uncover the patterns of gait features associated with increasing PD severities. This analysis also provided an unbiased and quantitative ranking of the degree of locomotor deficits in MPTP-treated monkeys, which could be readily exploited to assess the beneficial impact of therapeutic interventions such as L-Dopa administration. Our combined results highlight the striking sensitivity and rich information content of our recordings and analytic tools. This neuromotor analysis platform establishes the settings to evaluate safety and efficacy of therapeutic interventions to restore locomotion after PD and other neuromotor disorders.

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Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson's Disease

Support: NIH-NINDS Grant R01 NS059600

Center for Development and Behavioral Neuroscience at Binghamton University

Title: Modulation of primary motor cortex neurotransmitter signaling by dopamine lesion and L-DOPA therapy using a rat model of Parkinson's disease

Authors: *D. LINDENBACH, A. GOLDENBERG, C. OSTOCK, M. CONTI, C. BISHOP; Psychology, Binghamton Univ., Binghamton, NY

Abstract: Parkinson's disease (PD) is typically viewed as a movement disorder resulting from the death of cells that supply dopamine (DA) to the striatum. However, many aspects of motor cortex function are abnormal in PD patients and animal models of PD, implicating motor cortex involvement in disease symptoms and their treatment. We sought to characterize changes in primary motor cortex (M1) neurotransmitter signaling using the rat unilateral 6-hydroxdopamine model of PD. Animals were studied in their "off-drug" state as well as after treatment with the DA precursor L-DOPA (6 mg/kg). The first experiment investigated changes in tissue concentrations of M1 monoamines. At least 3 weeks after DA lesion surgery, tissue from striatum and M1 was dissected for analysis of dopamine, serotonin, and norepinephrine using high performance liquid chromatography. In the second set of experiments, we performed in vivo microdialysis of M1 to analyze extracellular concentrations of glutamate and GABA in real-time. Consistent with prior literature, treatment with L-DOPA improved movement in hemiparkinsonian rats, but caused the gradual development of L-DOPA-induced dyskinesias. Results from experiment 1 show that M1 tissue levels of DA are not reduced after 6-hydroxydopamine lesions despite a severe (~99%) loss of striatal DA. Preliminary data from experiment 2 suggest that L-DOPA increases M1 glutamate levels concomitant with L-DOPA-induced dyskinesia in DA-lesioned rats. Together, the data suggest that M1 neurotransmitter levels are dynamically modulated in the PD brain. Further investigations will shed light on the role of each system in controlling motor output.

Disclosures: D. Lindenbach: None. **A. Goldenberg:** None. **C. Ostock:** None. **M. Conti:** None. **C. Bishop:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 241.20/O3

Topic: C.04. Parkinson's Disease

Title: Cerebellar recruitment by subthalamic nucleus deep brain stimulation for Parkinson's disease

Authors: *A. C. SUTTON[1], J. G. PILITSIS[2,1], D. S. SHIN[1];
[1]Ctr. for Neuropharm. and Neurosci., Albany Med. Col., Albany, NY; [2]Neurosurg., Albany Med. Ctr., Albany, NY

Abstract: Deep brain stimulation (DBS) surgery for advanced Parkinson's disease (PD) has been performed nearly 100,000 times and is effective in managing motor symptoms of PD in well-selected individuals. However, expanding the patient population suitable for DBS and minimizing stimulation-induced side-effects are unmet needs which continue to encourage research into understanding the therapeutic effects of DBS; at present, the mechanisms of DBS remain unknown. Recently, efforts to examine circuit mechanisms of DBS have extended beyond the traditional basal ganglia (BG)-thalamus loop to include cortical and cerebellar circuits. Functional imaging has shown changes in cerebellar activity due to PD and DBS, yet the cerebellum has an intricate and complex microcircuitry that cannot be parsed by conventional imaging techniques. DBS for PD is most commonly applied to the subthalamic nucleus (STN), a BG nucleus that has recently been shown to have a disynaptic connection with the cerebellum. In our study, we assess whether STN-DBS provides efficacy by recruiting cerebellar activity and connected brain regions by applying STN-DBS in hemiparkinsonian rats and simultaneously recording from the STN, pedunculopontine tegmental nucleus (PPTg), Purkinje layer of cerebellar cortex, deep cerebellar nuclei (DCN), and ventromedial nucleus of the thalamus (VM). While STN neurons decreased spiking activity during DBS, a concomitant decrease was also noted in most PPTg and Purkinje neurons, presumably from reduced STN glutatmatergic signaling. In contrast, activity in the DCN and VM increased during DBS, which likely resulted from reduced inhibitory input from Purkinje cells. Then, as a proof-of-concept that STN-DBS recruits cerebellar activity to contribute to efficacy, we apply STN-DBS with sub-therapeutic current amplitude with a co-stimulation of the cerebellum to see if we can elicit similar improvement in forelimb akinesia as seen in hemiparkinsonian rats with supra-threshold current

stimulation alone. Notably, we found that this was the case and this novel finding demonstrates that STN-DBS modulates cerebellar activity to modify motor function. Our study is the first to characterize the changes in cerebellar activity in response to STN-DBS using electrophysiology and unmask a potential for stimulating the cerebellum to potentiate STN-DBS efficacy and/or minimize stimulation-induced adverse effects.

Disclosures: A.C. Sutton: None. **J.G. Pilitsis:** None. **D.S. Shin:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson's Disease

Support: NIH K08 NS 52232

NIH RO1 NS 7087

Title: Human intraoperative fMRI during STN DBS for Parkinson's Disease reveals BOLD activation in motor circuitry

Authors: *E. KNIGHT[1], K. M. WELKER[1], J. HUSTON, III[1], J. P. FELMLEE[1], D. A. CLAYTON[1], I. KIM[1], K. E. BENNET[1], C. D. BLAHA[2], K. H. LEE[1], S. CHANG[1]; [1]Mayo Clin., Rochester, MN; [2]Univ. of Memphis, Memphis, TN

Abstract: Although Deep Brain Stimulation (DBS) is widely performed for a variety of movement and psychiatric disorders, the effect of this therapy on the global neural circuitry is as yet poorly understood. fMRI has emerged as a powerful technique to visualize these circuitry effects; however, fMRI has traditionally not been performed in patients undergoing DBS due to safety concerns. Development of this technique would allow for visualization of the global circuitry responsible for the therapeutic effect of DBS, as well as potentially allow for the development of the use of fMRI as a biomarker of effective electrode placement and stimulation parameters. Therefore, we present the development of a methodology to allow for safe intraoperative fMRI during DBS surgery. This approach involves externalization of the DBS lead and application of 6 second periods of stimulation with 60 second rest periods using an external pulse generator while simultaneously acquiring gradient echo (GRE) echo planar imaging (EPI). All combinations of sequences and MR coils were first tested for safety by measurement of temperature change in an anthropomorphic phantom. Temperature increase was less than 0.2ºC. We then demonstrate the efficacy of this approach for obtaining functional

images during subthalamic nucleus (STN) DBS in patients with Parkinson's Disease to test the hypothesis that STN DBS would lead to blood oxygen level dependent (BOLD) signal increase in motor cortices. Consistent with our hypothesis, with left unilateral STN DBS at 2V 185Hz 90us, contact 0 negative and 3 positive, there was BOLD signal increase in the ipsilateral motor cortex as well as the ipsilateral thalamus, caudate, and cerebellum. Our results suggest that STN DBS results in modulation of motor circuitry, which may underlie the therapeutic effect of STN DBS in Parkinson's Disease.

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Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: C.04. Parkinson's Disease

Support: ANR-09-EMER-005

Title: A model of how transmission delays inside the basal ganglia proper cause beta-band oscillations in Parkinson's desease

Authors: *B. GIRARD[1], J. LIÉNARD[2,3];
[1]CNRS / UPMC, Paris Cedex, France; [2]ISIR, CNRS / UPMC, Paris, France; [3]Washington State Univ., Vancouver, WA

Abstract: In a previous study [1], we presented a mean-field model of the whole basal ganglia of monkeys, defined by a hundred parameters half of which where set according to experimental measurements from the literature, while the remaining ones were optimized in order to fit to both anatomical and electrophysiological data. We obtained a large set (more than one thousand) of relatively similar parameterizations compatible with all these constraints. The structure of these models does not rely on a strict segregation between direct/indirect pathways, as anatomical data suggest it is not the case in monkeys (e.g. [2]).
In these models, the transmission delays between basal ganglia nuclei were simplified. Here, we present a procedure by which we extract from the experimental literature (e.g. [3-8] and more) the delay configuration which is the most plausible. We then show that using this delay configuration with the previously obtained models, β-band oscillations appear when the activity of the STN, GPe, GPi and SNr increases (as a consequence of the decreased effect of their

inhibitory dopamine D2 receptors), while an imbalance in the direct indirect pathways is not necessary.
A sensitivity analysis on the critical pallido-subthalamic delays around their optimal values, shows that the slower θ-band oscillations can not possibly emerge in these basal ganglia proper models. This leads to the hypothesis that they could be caused by the longer delays of the cortico-baso-thalamo-cortical loops.
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[2] Parent, A., et al. (1995). Single striatofugal axons arborizing in both pallidal segments and in the substantia nigra in primates. Brain Res.
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[8] Iwamuro, et al. (2009). Organization of motor cortical inputs to the subthalamic nucleus in the monkey. The Basal Ganglia IX.

Disclosures: B. Girard: None. **J. Liénard:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson’s Disease

Title: Mapping DNA double-strand breaks in human and mouse brains

Authors: *G. TORRES[1], J. R. LEHESTE[2];
[2]Biomed. Sci., [1]NYIT COM, OLD WESTBURY, NY

Abstract: Neurons of the mammalian brain are susceptible to DNA damage following external (e.g., γ-radiation) and internal (e.g., α-synuclein deposit) insults. Indeed, it is thought that neuronal DNA damage may be responsible for the pathology often seen in neurodegenerative diseases such as Parkinson's disease (PD). Here we have mapped the parkinsonian brain and the brains of animal models of PD (e.g., Pit X3-1) with specific markers of DNA double-strand breaks. To accomplish this experimental goal, we have used two cellular markers of DNA damage: anti-phospho-histone H2A.X (Ser139) and 53BP1 (P-53 binding protein). Both of these markers label DNA damage in post-mitotic cells and can be used in brain pathology contexts. For labeling of neurons, we performed immunocytochemistry and Western blotting on human PD brains and adult PitX3-1 mouse brains. We report here that human and mouse brains show a significant number of labeled neurons positive for DNA double-strand breaks. Areas of the brain showing DNA double-strand breaks included the cortex, the hippocampus, the hypothalamus, the midbrain and cerebellum. Immunoreactivity focus for both markers (i.e., anti-phospho-histone H2A.X and 53BP1; P-53 binding protein) was exclusively nuclear and could easily be identified through light and confocal microscopy. This pattern of DNA labeling was also reproducible in vitro studies using human neuro-blastoma and neural-progenitor cells. It appears, therefore, that histone and chromatin modifications can accurately be identified and experimentally used for assessing neuronal DNA damage in the mammalian brain.

Disclosures: G. Torres: None. **J.R. Leheste:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson's Disease

Support: USPHSG NS34696

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JPB Foundation

Title: Corticostriatal synapses on striatopallidal neurons are lost in a mouse model of Parkinson's disease and restored in mice exhibiting L-DOPA-induced dyskinesia

Authors: *S. M. GRAVES[1], L. E. SEBEL[1], J. L. PLOTKIN[1], T. FIEBLINGER[2], M. A. CENCI[2], D. J. SURMEIER[1];
[1]Physiol., Northwestern Univ., Chicago, IL; [2]Dept of Exp. Med. Sci., Lund Univ., Lund, Sweden

Abstract: A hallmark of Parkinson's disease (PD) is dopamine depletion of the striatum. L-DOPA, the dopamine precursor, treats the motor symptoms of PD but long-term therapy is complicated by L-DOPA-induced dyskinesia (LID). Numerous reports indicate an involvement of direct pathway spiny projection neurons (dSPNs) in LID (Cenci, *Trends Neurosci* 30: 236-43, 2007; Guigoni and Bezard, *Parkinsonism Relat Disord*. 3:S64, 2009) but it is unclear whether there also is participation of indirect pathway spiny projection neurons (iSPNs). To examine this issue, hemizygous BAC-D2-eGFP mice were unilaterally lesioned using 6-OHDA injections into the medial forebrain bundle and then administered L-DOPA (6-12 mg/kg/day i.p.) or saline on alternate days for 10 days. This L-DOPA regimen rendered mice dyskinetic. As previously described (Day *et al.*, *Nat Neurosci.* 9(2): 251, 2006), iSPNs from lesioned mice that were not given L-DOPA lost spines. However, in iSPNs from dyskinetic mice treated with L-DOPA, spine density was elevated and near normal levels. In contrast, iSPN dendrites in lesioned mice were fewer in number and shorter, regardless of whether mice were saline and L-DOPA treated. To determine the identity of the synaptic connections lost with lesioning and restored by L-DOPA treatment, optogenetic circuit mapping was employed in transgenic mice expressing channel rhodopsin 2 in the cortex. After 6-OHDA lesioning, iSPNs lost cortical axospinous synapses (~70% in control *vs*. ~50% in dopamine depleted). The percent of cortically responsive spines rose in dyskinetic mice (~70%), suggesting that the newly formed spines received cortical input. These results suggest that the loss of cortical connectivity with iSPNs following 6-OHDA lesioning is restored following L-DOPA treatment. The association of this restoration in connectivity with dyskinesia could mean that this connectivity is abnormal and leads to inappropriate indirect pathway activity and the failure to appropriately suppress unwanted dyskinetic movements.

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Poster

241. **Parkinson's Disease: Circuit Mechanisms**

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Topic: C.04. Parkinson's Disease

Support: CIHR Grant MOP-115008

Title: Striatal projection neurons of the D1 and D2 types are differentially altered in a mice model of Parkinson's disease

Authors: M. G. SÁNCHEZ, C. BORIES, D. GAGNON, Y. DE KONINCK, J. M. BEAULIEU, A. PARENT, *M. PARENT;
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Abstract: The main neuropathological hallmark of Parkinson's disease is a progressive neuronal loss of the substantia nigra pars compacta, which causes a marked decrease of the striatal dopaminergic innervation. The activity of striatal projection neurons expressing dopaminergic receptors of the D_1 type is known to be decreased as a functional consequence of such dopaminergic depletion compared to striatal neurons expressing D_2 receptors. However, the specific consequences of a dopaminergic striatal denervation on fine dendritic and spine processes belonging to striatal neurons remains poorly known. Therefore, we looked for possible morphological changes at the level of three different types of striatal projection neurons: the D_1+, the D_2+ and the D_1+/D_2+ medium spiny neurons, after dopaminergic denervation. We used double transgenic BAC mice *Drd1a*-tdTomato/*Drd2*-EGFP, a model that allows a clear identification of the direct and indirect striatal projection neurons based on their dopamine receptor content. Striatal dopaminergic denervation was induced by unilateral injections of 6-hydroxydopamine in the medial forebrain bundle. Cylinder test and immunohistochemistry staining of tyrosine hydroxylase and dopamine transporter confirmed the extent of the lesion. Subsequently, the D_1+, D_2+ and the D_1+/D_2+ striatal neurons were subjected to single-cell intraneuronal injections of Lucifer yellow combined to immunohistochemistry. Detailed morphometric analyses of dendritic trees and spines as well as their glutamatergic afferents were performed on high-resolution images acquired from a confocal microscope. Our results indicate that the dopaminergic denervation causes a decrease in the total dendritic length of the D_1+ and D_2+ medium spiny projection neurons analyzed. On the lesioned side of the brain, spine density was increased along the dendrites of the D_1+ striatal neurons and decreased on the D_2+ neurons, compared to the intact side. No significant changes of spine density were detected along the dendrites of the D_1+/D_2+ medium spiny neurons. These results provide direct evidence for differential morphological alterations of D_1+ and D_2+ striatofugal neurons involved in the direct and indirect pathways, respectively, following dopaminergic denervation of the striatum. Our preliminary results indicate that other factors, such as a reorganization of the corticostriatal glutamatergic projections might also contribute to the neuroadaptative changes reported above.

Disclosures: M.G. Sánchez: None. **C. Bories:** None. **D. Gagnon:** None. **Y. De Koninck:** None. **J.M. Beaulieu:** None. **A. Parent:** None. **M. Parent:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 241.26/O9

Topic: C.04. Parkinson's Disease

Support: EraNet Neuron

Title: Disturbed development and stability of dendritic spines of adult-born olfactory bulb neurons in A30P α-synuclein mice

Authors: *J. NEUNER[1], S. V. OVSEPIAN[2], S. FILSER[2], M. DOROSTKAR[1], J. HERMS[2]; [1]Ludwig-Maximilians-University, München, Germany; [2]DZNE, München, Germany

Abstract: Impaired olfaction is an early symptom in Parkinson disease (PD) with yet unknown underlying mechanisms. Here, we examined the possible link between the PD-related α-Synuclein (α-SYN) pathology, olfactory deficits and the integration of adult-born granule cells (GC) into the neuronal circuitry of the olfactory bulb (OB), using A30P α-SYN mice. We report that accumulation of α-SYN in mitral cells (MC) strongly interferes with the olfactory discrimination as well as integration of the adult-born GCs into the neuronal networks, with detrimental effects on the survival of new arrivals. Through combining chronic in vivo two-photon imaging with time-coded labelling of newborn GCs, we demonstrate for the first time a considerable impairment of the development and stability of dendritic spines of adult-born GCs, resultant in a significantly decreased lifespan of the nascent spines with an overall reduction of their density in the adult OB of α-SYN mice, as compared to wild-type controls. Impaired spine development of GCs is maturation stage-dependent, progresses with age and appears to be contributed by the occurrence of the α-SYN pathology in MCs. Our data suggest the impaired signalling between α-SYN enriched MCs and adult-born GCs as principal causative to the compromised integration of the latter into the pre-existing networks of the OB and to their decreased survival. Hence, important novel clues are provided on the role of dendrodendritic synapses between MCs and GCs in the maintenance and plasticity of existing circuits in the adult brain, in addition to their well recognized functions in olfactory discrimination via lateral inhibition.

Disclosures: J. Neuner: None. **S.V. Ovsepian:** None. **S. Filser:** None. **M. Dorostkar:** None. **J. Herms:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 241.27/O10

Topic: C.04. Parkinson's Disease

Support: NIH Grant NS068231

Title: Physiological changes in the PPN in a normal and parkinsonian non-human primate during rest and gait

Authors: *H. M. HUDSON[1], A. T. CONNOLLY[2], J. ZHANG[1], K. B. BAKER[1], J. L. VITEK[1]; [1]Neurol., [2]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: The pedunculopontine nucleus (PPN) is being investigated in patients with Parkinson's disease (PD) as a potential therapeutic target for the treatment of gait and posture impairments that are refractory to current medical and surgical interventions. Recent trials of PPN deep brain stimulation (DBS) have been inconsistent, however. In contrast to the higher pulse frequencies used during therapeutic globus pallidus (GPi) or subthalamic nucleus (STN) DBS, PPN DBS typically involves the application of lower frequencies (~10 - 20 Hz). While the therapeutic benefit of high frequency GPi and STN DBS, as well as levodopa replacement therapy, is thought to derive at least in part from the disruption of excessive and pathological low frequency oscillations present in the basal ganglia-thalamo-cortical loop, the benefits of low frequency stimulation of PPN are thought to arise from the augmentation of physiological synchronizations that contribute to normal movement. This study was undertaken to characterize the changes in neuronal activity (single unit and local field potentials) in the PPN in the parkinsonian state, with and without dopamine, and assess the effect of PPN DBS on gait mechanics using our treadmill-based model of the ambulating non-human primate. Additionally, we report here local field potential data recorded from an 8-channel, scaled DBS lead implanted in the area of the PPN of one non-human primate. Recordings were taken with the animal at rest and during ambulation on a motorized treadmill in the naïve as well as the moderate parkinsonian state (induced by systemic injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), with and without levodopa replacement medication. We will elucidate the relative changes in spectral content across the primary electroencephalographic frequency bands (i.e., delta, theta, alpha, beta and gamma) as a function of parkinsonian state and address the hypothesis that low frequency oscillations are associated with normal movement and are reduced in a parkinsonian state.

Disclosures: H.M. Hudson: None. **A.T. Connolly:** None. **J. Zhang:** None. **K.B. Baker:** None. **J.L. Vitek:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 241.28/O11

Topic: C.04. Parkinson's Disease

Support: The Korea Healthcare technology R&D Project, Ministry of Health and Welfare, Republic of Korea (A092052)

The project of Global Ph.D. Fellowship which National Research Foundation of Korea conducts from 2012 (2012004325)

Title: Low frequency stimulation of pedunculopontine nucleus modulates hyperactivity of basal ganglia in the parkinsonian rat models

Authors: *E. PARK[1], I. SONG[2], D. JANG[1], I. KIM[1];
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Abstract: Deep brain stimulation (DBS) is an established therapy for movement disorders. The subthalamic nucleus (STN) or globus pallidus internus (GPi) is considered an effective therapeutic target for Parkinson's disease. Although STN or GPi DBS produce clinical improvement in motor symptoms such as tremor, rigidity and bradykinesia, it responds less in gait disturbance and postural instability for advanced Parkinson's disease. Recently, the pedunculopontine nucleus (PPN; the equivalent of the pedunculopontine tegmental nucleus (PPTg) in rodent) has been introduced as an alternative therapeutic target for advanced Parkinson's disease with severe and medically intractable axial symptoms like gait and postural impairment. The PPN is reciprocally connected to the basal ganglia such as STN, GPi and substantia nigra, and projected to the thalamus and spinal cord. Specially, the PPN receives GABAnergic afferents from output structures of basal ganglia such as the substantia nigra par reticulate (SNr), entopeduncular nucleus (EP; GPi in primates) and striatum as well as glutamatergic afferents from the STN and frontal cortex. The PPN also sends excitatory efferents to the substantia nigra pars compacta (SNc), SNr, EP and STN. Therefore, the PPN performs the potential role of controlling the basal ganglia output and plays an important role in the initiation, maintenance and modulation of gait and postural stability. The current study aims to investigate the synchronized changes in neuronal activity of both STN and SNr with low frequency stimulation of PPTg in intact as well as in rat models of Parkinson's disease, and to provide a comparison of analyzed data from both intact and rat models with Parkinson's disease. Thirty male Sprague-Dawley rats (180-200 g) were used in this study. The animals were randomly divided into 2 groups: intact control group and 6-OHDA lesioned group. The 6-OHDA lesioned group was treated with unilateral 6-OHDA injections. The implantation of electrodes was performed 4 weeks after 6-OHDA lesion of SNc under anesthesia. The stimulation electrode was implanted into the PPTg and the two microelectrodes were used for electrophysiological recordings. In this research, we found that the two major structures of the basal ganglia, which are STN and SNr, has hyperactivity after 6-OHDA lesion. However, low frequency stimulation

of PPTg reverses the firing rate of two structures into normalization. These results can be used to evaluate the role of low frequency stimulation of PPTg with the recorded responses in basal ganglia.

Disclosures: E. Park: None. **I. Song:** None. **D. Jang:** None. **I. Kim:** None.

Poster

241. **Parkinson's Disease: Circuit Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 241.29/O12

Topic: C.04. Parkinson's Disease

Title: Selective inactivation of FosB expressing neurons in the juxtacapsular BST decreases L-Dopa induced dyskinesia

Authors: *P.-O. FERNAGUT[1,2], M. BASTIDE[1,2], C. GLANGETAS[3], C. GROSS[1,2], F. GEORGES[3], E. BEZARD[1,2];
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Abstract: The most effective symptomatic therapy of Parkinson's disease (PD) remains the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA). Long-term treatment leads to involuntary aimless movements called L-DOPA-induced dyskinesia (LID). Accumulation of the truncated splice variant of FosB (ΔFosB) in the striatum has been established as a surrogate marker of LID in rodent and primate experimental models. Other dopaminoceptive structures however also display an L-dopa-driven overexpression of ΔFosB. The juxtacapsular part of the Bed Nucleus of the Stria Terminalis (jBST) is a limbic relay of basal ganglia information that display dramatic increase in immediate-early gene expression in response to L-dopa only in dyskinetic animals. Furthermore, the BST was found to be metabolically active in dyskinetic monkeys and not in non-dyskineic ones, rasing the possibility that this nucleus plays a role in LID manifestation.
To assess if ΔFosB expressing neurons in the jBST have an impact on LID, we thus targeted these neurons using the selective Daun02/β-galactosidase inactivation method. β-galactosidase expression, driven by a FosB promoter, was achieved using a FosB-Lacz lentiviral vector injected in the jBST of unilaterally 6-hydroxydopamine lesioned rats. Following chronic L-Dopa treatment (6mg/kg) and the induction of LID, rats were injected with Daun02 in the jBST. Three days after Daun02 administration, animals were tested daily with L-Dopa to assess LID and L-

Dopa-induced rotations. Inactivation of FosB-expressing neurons significantly reduced LID (-20,3%, P<0,05) but had no impact on L-Dopa-induced rotations. This study is the first showing the impact of selective inactivation of ΔFosB expressing neurons in LID outside the basal ganglia.

Disclosures: P. Fernagut: None. **E. Bezard:** None. **M. Bastide:** None. **C. Gross:** None. **C. Glangetas:** None. **F. Georges:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.01/P1

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Funded by Cure Huntington's Disease Initiative (CHDI)

Pfizer Inc., provided compounds and dose formulation information for this study.

Animals were generated by Dr Hoa Nguyen, University of Tubingen, Germany.

Title: Impact of phosphodiesterase 9A and 10A inhibition on aberrant cortically-evoked spike activity in the striatum of transgenic rats that model Huntington's disease

Authors: *A. R. WEST[1], S. CHAKROBORTY[1], A. M. DEC[1], C. J. SCHMIDT[2];
[1]Rosalind Franklin Univ. Med. Sci., NORTH CHICAGO, IL; [2]Pfizer Inc., Cambridge, MA

Abstract: Huntington's disease (HD) is an autosomal dominant disorder associated with abnormal expansion in CAG trinucleotide repeats within the HD gene and degeneration of striatal medium-sized spiny projection neurons (MSNs). Recent studies indicate that the metabolism of cyclic nucleotides by phosphodiesterases (PDEs) is likely to be perturbed in MSNs in the HD striatum. Studies providing evidence for both decreased cAMP/cGMP production and deficits in corticostriatal transmission in advanced HD suggest a link between cyclic nucleotide signaling and disrupted MSN network activity. The current study assessed cortically-evoked firing in aged (8-10 months old) wild-type (WT) and full-length BAC transgenic line 5 HD rats (TG5) treated with vehicle, the PDE9A inhibitor PF-4447943, or the PDE10A inhibitor PF-2545920. WT and TG5 rats were anesthetized with urethane and single-unit spike activity was isolated during low frequency electrical stimulation (0.5 Hz, 0.5 msec, 0.4-1.0 mA) of the ipsilateral motor cortex. Compared to WT rats, TG5 animals exhibited decreased spike probability during cortical stimulation delivered at lower stimulation intensities

(0.6 mA). No differences in cortically-evoked spiking were observed at higher stimulus intensities (≥ 0.8 mA) and spike onset latency was similar across groups and intensities. Systemic administration of both the PDE9A inhibitor PF-4447943 (3.2 mg/kg, s.c.) and the PDE10A inhibitor PF-2545920 (1 mg/kg, s.c.) significantly decreased the onset latency of cortically-evoked spikes at all stimulation intensities in WT rats as compared to vehicle-treated controls. Systemic administration of PF-4447943 also decreased the onset latency of cortically-evoked spikes in TG5 rats and reversed deficits in spike probability observed in these animals. Interestingly, PF-2545920 administration had only modest facilitatory effects on onset latency in TG5 rats and no effects on spike probability. Robust increases in cortically-evoked spike activity were observed in MSNs recorded in WT rats, thus it is likely that PDE10A function is compromised in the HD striatum. Given that PDE10A metabolizes both cAMP and cGMP, while PDE9A is specific for cGMP, it is possible that MSNs in the aged HD rat striatum lose their ability to respond to transmitters that signal through the cAMP/PKA pathways (e.g., dopamine, adenosine) but not cGMP (e.g., nitric oxide). Thus, drugs such as PF-4447943 which act to facilitate cGMP signaling could be useful therapeutic agents for restoring corticostriatal transmission in HD, and potentially, alleviating motor and cognitive symptoms of this disease.

Disclosures: A.R. West: None. **S. Chakroborty:** None. **A.M. Dec:** None. **C.J. Schmidt:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.02/P2

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: This work was funded by CHDI

The BACHD rats were developed by Dr. Hoa Nguyen, University of Tubingen, Germany

MP-10 was provided by Pfizer Inc

Experiments were conducted by PsychoGenics in consultation with CHDI and Pfizer.

Title: Evaluation of MP-10, a PDE10 inhibitor, on BACHD transgenic rats using dual recording of single units in Globus Pallidus and Subthalamic nucleus

Authors: S. ZHONG[1], G. TOMBAUGH[1], L. MRZLJAK[2], V. BEAUMONT[2], C. SCHMIDT[3], M. ZALESKA[3], C. CHANG[3], *A. GHAVAMI[1];

[1]Psychogenics, Montvale, NJ; [2]CHDI Management/Foundation, Los Angeles, CA; [3]Pfizer, Cambridge, MA

Abstract: Chorea in Huntington's disease (HD) patients might be due in part to a dysfunction of the indirect pathway (IP) of the basal ganglia. D2-expressing striatal medium spiny neurons (MSN), giving rise to IP projections, appear more vulnerable to expression of mutant huntingtin (mHtt). A consequence of the preferential loss of striato-external pallidal (GPe) projections in HD patients would be expected to result in increased GPe firing rate, reduced STN firing rate, reduced activity of GPi, and ultimately overactivity of the thalamus, resulting in chorea. Previous studies in the mouse BACHD model (6 month old), reported an age-dependent increase in mean firing rate of GP neurons and decrease in the mean firing rate of STN neurons in vitro (D.J. Surmeier, Northwestern Univ.) and in vivo (James Tepper, Rutgers Univ.). The current study, using dual simultaneously recording from GP and STN, demonstrated that comparable alterations in firing rates was also detected in 8 to 15 months old BACHD full length transgenic rats. Mean spontaneous firing rates showed a significant increase in GPe (22.2±2.0 Hz in WT vs. 30.4±2.5 Hz in BACHD, $P<0.02$) and a significant decrease in STN (10.8±1.1 Hz in WT vs. 6.7±1.0 Hz in BACHD, $P<0.005$). The phosphodiesterase 10 (PDE10) is highly expressed within dopaminoreceptive MSNs of the striatum and PDE10 inhibitors have been viewed as a potential treatment for schizophrenia. To provide a rationale for developing PDE10 inhibitors as a therapy for HD, we evaluated whether MP-10, a specific and potent PDE10 inhibitor, would be able to reverse the altered firing rate observed in BACHD rat. A single bolus IV injection of MP-10 at all doses studied (0.18, 0.52, and 1.5 mg/kg) produced a clear increase in the firing rate of STN neurons in both WT and TG rats. Surprisingly, MP-10 effect on the GP firing rate was modest. PK/PD relationship was studied by collecting blood samples at 5, 30, 60, and 120 minutes after compound administration. The earliest effect on firing rates was observed at 3 to 5 minutes post MP-10 administration, which may reflect the time needed for cAMP/cGMP to reach a critical level. The magnitude of firing rate increase in STN was not dramatically different across doses, but the 0.52 and 1.5mg/kg doses produced longer lasting effects than the 0.18mg/kg dose. Our data provide evidence that are complementary to the prevailing hypothesis in HD patients; expression of mHtt in rats alters the firing properties of neurons in the "indirect" pallidosubthalamic pathway. MP-10 restored the low STN firing rates in BACHD rats, which is consistent with a potential therapeutic action of PDE10 inhibitors for the treatment of HD.

Disclosures: S. Zhong: A. Employment/Salary (full or part-time):; PsychoGenics. 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.; CHDI foundation. **G. Tombaugh:** A. Employment/Salary (full or part-time):; PsychoGenics. 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.; CHDI foundation. **L. Mrzljak:** A. Employment/Salary (full or part-

time):; CHDI. **V. Beaumont:** A. Employment/Salary (full or part-time):; CHDI. **C. Schmidt:** A. Employment/Salary (full or part-time):; Pfizer. **M. Zaleska:** A. Employment/Salary (full or part-time):; Pfizer. **C. Chang:** A. Employment/Salary (full or part-time):; Pfizer. **A. Ghavami:** A. Employment/Salary (full or part-time):; PsychoGenics. 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.; CHDI foundation.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.03/P3

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Selecting a proof of concept tool to test the effects of TrkB activation in Huntington's disease models

Authors: G. MCALLISTER[1], D. TODD[1], M. WALL[1], I. GOWERS[1], S. PENROSE[1], G. WISHART[1], I. MUNOZ-SANJUAN[3], C. DOMINGUEZ[3], J. ARJOMAND[4], M. LAMERS[1], J. TURNEY[1], R. GRISHANIN[5], J. PAULSEN[6], J. C. LIN[5], G. FLYNN[7], S. FRATANTONI[7], D. FISCHER[2], S. DIJKSTRA[7], J. WITYAK[3], *J. A. BARD[4];
[1]BioFocus, Saffron Walden, United Kingdom; [2]BioFocus, Leiden, Netherlands; [3]CHDI, Los Angeles, CA; [4]CHDI, PRINCETON, NJ; [5]Pfizer, South San Francisco, CA; [6]Pfizer, Cambridge, MA; [7]Galapagos, Leiden, Netherlands

Abstract: Huntington's disease (HD) is a devastating, genetic neurodegenerative disease caused by a tri-nucleotide expansion in exon 1 of the huntingtin gene. HD is clinically characterized by chorea, mood and psychiatric disturbance and cognitive deficits with later symptoms including rigidity and dementia. Pathologically, the cortico-striatal pathway is severely damaged, resulting in striatal and cortical atrophy in late-stage disease. Brain-derived neurotrophic factor (BDNF) is a neuroprotective, secreted protein which binds with high affinity to the extracellular domain of the tropomyosin related kinase B (TrkB) receptor promoting neuronal cell survival by activating the receptor and its down-stream signaling cascade. Reduced levels of BDNF produced by cortical neurons and released in the striatum have been implicated in the pathogenesis of Huntington's disease (HD) and therefore, the ability to enhance TrkB signaling using a BDNF mimetic may slow or reverse HD onset. Using recombinant and native assay formats, we have evaluated a panel of small molecules and functional antibodies, reported to be TrkB agonists, and identified the best candidate for future *in vivo* proof of concept studies in HD models.

Disclosures: G. McAllister: None. **D. Todd:** None. **M. Wall:** None. **I. Gowers:** None. **S. Penrose:** None. **G. Wishart:** None. **I. Munoz-Sanjuan:** None. **C. Dominguez:** None. **J. Arjomand:** None. **M. Lamers:** None. **J. Turney:** None. **R. Grishanin:** None. **J. Paulsen:** None. **J.C. Lin:** None. **G. Flynn:** None. **S. Fratantoni:** None. **D. Fischer:** None. **S. Dijkstra:** None. **J. Wityak:** None. **J.A. Bard:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.04/P4

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Atg4b-dependent autophagic flux alleviates huntington's disease progression

Authors: *C. C. PROENCA, N. STOEHR, M. BERNHARD, T. BOUWMEESTER, I. GALIMBERTI;
Novartis Pharma NIBR, Basel, Switzerland

Abstract: Huntington's disease (HD) is characterized by an abnormal accumulation of mutant huntingtin protein (mHtt) in inclusion bodies, leading to a selective degeneration of striatal and cortical neurons. Despite the ubiquitous expression of Htt throughout the body, the reason for this selectivity remains elusive. Here we have developed a new model to study medium-sized spiny neuron (MSN) degeneration in the context of HD. We produced organotypic cortico-striatal slice cultures (CStS) from HD transgenic mice mimicking specific features of HD progression. We were able to rescue HD progression and MSN degeneration in vitro by inducing autophagy using a catalytic inhibitor of the mTOR pathway. On the other hand, blocking autophagy by manipulating Atg4b accelerated disease progression. Finally, catalytic mTOR inhibition was inefficient in the presence of Atg4b overexpression, indicating that Atg4b is a key downstream player of mTOR.
These results establish modulators of Atg4b-dependent autophagic flux as new potential targets in the treatment of HD.

Disclosures: C.C. Proenca: None. **N. Stoehr:** None. **M. Bernhard:** None. **T. Bouwmeester:** None. **I. Galimberti:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.05/P5

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS-57722 (AR)

NIH Grant NS-28721 (AR)

The Methodist Hospitals Endowed Professorship in Neuroscience (AR)

NIH Grant EY-14998 (KRJ)

Title: Cerebral cortex develops normally in mice with early embryonic deletion of huntington from cortical pyramidal neurons

Authors: *I. DRAGATSIS[1], P. DIETRICH[1], Y. DENG[2], N. DEL MAR[2], J. ROGERS[2], M. J. IRUDAYAM[1], K. R. JONES[3], A. REINER[2];
[1]Physiol., [2]Anat. & Neurobio., Univ. of Tennessee/Health Sci. Ctr., Memphis, TN; [3]Molecular, Cellular, & Developmental Biol., Univ. of Colorado, Denver, CO

Abstract: Understanding the normal function of the Huntingtin gene is important in elucidating how its mutation leads to Huntington's disease. Studies have suggested that the wild-type huntingtin (htt) protein plays a critical role in neurogenesis and neuronal migration during cortical development (Godin et al., Neuron, 2010; Tong et al., J Neurosci, 2011). We therefore evaluated the impact of early embryonic deletion of htt from cortical pyramidal neurons, by using the cre-loxP system to inactivate the mouse htt gene (Hdh) in Emx1-expressing cell lineages. Since Emx1 is expressed in the pallial proliferative zone beginning at about E8, Emx1-Cre/+; Hdh flox/- mice sustain htt deletion from cortical pyramidal neuron and glial precursors and their progeny. Western blot analysis confirmed reduction of htt expression in the cortex of Emx1-Cre/+; Hdh flox/- mice, with residual cortical htt expression in all likelihood restricted to cortical interneurons of the subpallial lineage and/or endothelial cells of the vasculature. Histological analyses showed that despite the loss of htt from early in development, cortical thickness and lamination were normal. Immunocytochemical evaluation of layer-specific cell populations such as calbindinergic neurons in layers 2-3, VGLUT2+ fibers in layer 4, SMI32+ and CTIP2+ neurons in layer 5, and FoxP2+ neurons in layer 6 revealed no obvious laminar abnormalities. Control and Emx1-Cre/+; Hdh flox/- mice were studied behaviorally and histologically over a 7-24 month age range to determine if absence of htt from cortical pyramidal neurons yields progressive abnormalities. Emx1-Cre/+; Hdh flox/- mice were slightly impaired in rotarod performance, and hyperactive compared to WT in an open field test at all ages. Emx1-Cre/+; Hdh flox/- mice also showed a greater age-related decline in locomotor speed in open

field than WT mice. The only progressive neuropathology observed in cortex was increased astrocytic GFAP labeling in cortical layer 4. The striatum, globus pallidus and striatal projection systems were normal in morphology and neurochemistry. Our results show that embryonic deletion of huntingtin from developing pallium does not alter cortical neurogenesis or neuronal migration of cortical neurons to their correct laminar destinations, or yield major progressive pathology in adult mice. Early deletion of huntingtin from cortical neurons does, however, cause developmental or functional abnormalities that manifest as hyperactivity and modest motor impairments.

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Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.06/P6

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI

NINDS16375

Title: Post-translational modifications of huntingtin protein in HD brain

Authors: *T. RATOVITSKI[1], E. CHIGHLADZE[1], A. ZAVALA[1], E. WATKIN[1], N. ARBEZ[1], R. CHAERKADY[2], X. WANG[1], O. PLETNIKOVA[3], J. C. TRANCOSO[3], R. N. COLE[2], C. A. ROSS[1];
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Abstract: Post-translational modifications (PTMs) of expanded Htt are likely to be important mediators or modulators of HD pathogenesis. A number of PTMs of Htt have been identified, mainly within its N-terminal region. However, huntingtin is a very large multifunctional protein, providing a scaffold for regulated binding of other proteins. It is highly likely to have many sites of PTM. While there are a number of groups studying PTMs using cell models, there has been considerably less attention to identification of such modifications in HD mouse models and in human post-mortem HD brain. None of the previous studies were designed to characterize Htt PTMs systematically in the context of full-length normal and polyQ-expanded Htt endogenously expressed *in vivo* in HD mouse models and in HD human brain.

Here we used immunoprecipitation to purify full-length Htt from brain of homozygote HD KI175Q mice and human post-mortem brain and employed mass spectrometry to identify Htt PTMs, especially phosphorylation.
We have developed a large-scale Htt purification procedure to obtain sufficient amounts of purified Htt from mouse and human brain, detectable on the gel using protein stains. Quality control of samples showed the preservation of S13/S16 phosphorylation during the procedure. We were able to identify 25 Htt PTMs in the brains of either/or both KI 175Q and WT mice at 6 months of age. Among these, there are 3 lysine acetylation sites and 2 threonine phosphorylation sites. Out of 20 serine phosphorylation sites, 15 are novel, and 5 have been previously reported. In a parallel study we have identified a novel phosphorylation site S116 using HEK293 cells transiently transfected with human Htt (first 511 amino acid). This site was functionally validated in primary neurons: the S116A alteration showed significant and reproducible protection in the nuclear condensation assay.
For our studies in human brain, we have selected 3 HD cases and 3 controls based on an adequate purification of intact full-length Htt, and on the preservation of known S13/S16 phosphorylation sites in post-mortem material. We have achieved 56% coverage of WT Htt sequence, and 68% coverage of expanded Htt using trypsin for digestion, and have identified 11 Htt PTMs from the frontal cortex of human brain Among these are the previously reported S419/ S421 phosphorylation sites, which we had not detected in mouse brain. The other 9 PTMs are novel, and include 3 lysine acetylation sites and 6 phosphorylated serines.

Disclosures: T. Ratovitski: None. **E. Chighladze:** None. **A. Zavala:** None. **E. Watkin:** None. **N. Arbez:** None. **R. Chaerkady:** None. **X. Wang:** None. **O. Pletnikova:** None. **J.C. Trancoso:** None. **R.N. Cole:** None. **C.A. Ross:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.07/P7

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NS-57722 (AR)

the Cure Huntington’s Disease Initiative(AR) Foundation

The Methodist Hospitals Endowed Professorship in Neuroscience (AR)

Title: Pattern of premanifest loss of thalamostriatal and corticostriatal input to striatal projection neuron types in Q140 Huntington’s disease knock-in mice

Authors: *Y. DENG, T. WONG, A. REINER;
Univ. of Tennessee HSC, MEMPHIS, TN

Abstract: Motor slowing, forebrain white matter loss, and striatal shrinkage have been reported in premanifest Huntington's disease (HD) prior to overt striatal neuron loss. These findings raise the possibility that the earliest motor defects in HD victims may be related to the loss of excitatory cortical and thalamal afferent connectivity of motor striatum. In a prior study, we showed that in premanifest Q140 heterozygous HD knock-in mice not yet showing striatal projection neuron pathology, a 20% loss of thalamostriatal axospinous terminals is evident by 4 months of age, and a 30% loss of corticostriatal axospinous terminals is evident by 12 months of age. In the present study, we characterized the relative loss of thalamostriatal and corticostriatal axospinous terminals from direct pathway (D1+) and indirect pathway (D1-) striatal projection neurons, using immunolabeling to identify thalamostriatal (VGLUT2+) and corticostriatal (VGLUT1+) axospinous terminals. Measuring the size and abundance of such terminals on D1+ versus D1- spines to construct terminal size frequency distributions, we found that in wildtype mouse, VGLUT1 axospinous terminals on direct pathway neurons (D1+) showed a unimodal distribution with a peak at 0.5 µm (presumed to be IT-type corticostriatal terminals), while those on indirect pathway neurons (D1-) showed a mixture of small presumptive IT-type (≤0.6 µm) and large presumptive PT-type (>0.6 µm) axospinous terminals (Reiner et al., Frontiers 2010). The loss of corticostriatal terminals at 12 months of age in Q140 mice was preferentially on D1+ spines (70% reduction), and especially included small terminals (≤ 0.6 µm). Indirect pathway D1- spines showed slight loss of 0.6 µm terminals, and a mild loss of 0.9 µm terminals. For the thalamostriatal projection in WT mice, VGLUT2 terminals on D1+ spines showed a unimodal distribution with a peak at 0.4 µm, while those on D1- spines showed peaks at 0.3 µm and another at 0.5 µm). Thalamostriatal terminal loss in Q140 mice for both D1+ and D1- spines was comparable at 4 months and 12 months of age, and involved smaller terminals. Regression analysis showed that numerous open field motor parameters such as speed and length of locomotor segments were highly correlated with the spatial abundance of VGLUT1+ terminals on D1+ spines at 12 months, indicating the loss of corticostriatal terminals to D1 direct pathway neurons appeared to drive motor impairments in the Q140 mice at this age. Our results in general raise the possibility that cortical and thalamic input loss is an early event in human HD and contributes to early HD symptoms.

Disclosures: Y. Deng: None. **T. Wong:** None. **A. Reiner:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

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Program#/Poster#: 242.08/P8

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III, (grant numbers PI10/01072 and RETICS: RD06/0010/0006)

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Generalitat de Catalunya (grant number 2009SGR-00326)

Generalitat de Catalunya (AGAUR ST067914)

Title: Blockade of the Protein Kinase A pathway improves cognitive deficits in a Huntington's disease mouse model

Authors: S. Z. TYEBJI[1,2,3], A. SAAVEDRA[1,2,3], A. GIRALT[1,2,3], J. ALBERCH[1,2,3], *E. PEREZ-NAVARRO[1,2,3];
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Abstract: A delicate balance between the activities of kinases and phosphatases is required to maintain normal memory and plasticity processes. cAMP-dependent protein kinase (PKA) promotes the formation and maintenance of long-term memory (LTM) but an aberrant and sustained activation of this pathway can lead to memory dysfunction and alterations in long-term potentiation (LTP) in the hippocampus. We have previously shown that increased PKA activity is implicated in the cognitive impairment observed in Huntington's disease (HD) and that its regulation could be a promising therapeutic approach. Dopamine D1 receptor (D1R) and adenosine A2A receptor (A2AR) are G protein coupled receptors that stimulate PKA activity via cAMP production. Although we found that these receptor levels remain unchanged in the hippocampus of the R6/1 mouse model of HD, higher receptor sensitivity in the presence of mutant huntingtin could lead to increased PKA signaling. Here, we pharmacologically antagonized these receptors in order to regulate PKA signaling in the hippocampus. Twelve-week-old wild-type (WT) and R6/1 mice were chronically treated (daily intraperitoneal injections) with vehicle or SCH23390 (D1R antagonist) + SCH58261 (A2AR antagonist). Mice performed the novel object recognition test (NORT) at 13 weeks of age, the T-maze spontaneous alteration test (TSAT) at 14 weeks of age, and the passive avoidance (PA) task at 15 weeks of age. R6/1 mice treated with D1R and A2AR antagonists showed a significant improvement in cognitive performance whereas cognitive function was not altered in WT mice. This improvement correlated with a decrease (to WT levels) of the phosphorylation level of PKA substrates in the hippocampus of R6/1 mice while no changes were detected in WT mice. In contrast, chronic treatment with D1R or A2AR antagonist alone did not improve learning and memory in R6/1 mice. Interestingly, an acute blockade of these receptors 90-120 min before

training lead to significant LTM deficits in WT mice as assessed using the NORT, TSAT and PA tasks, but had no further effect on R6/1 mice. In conclusion, our results indicate that a combined chronic inhibition of hippocampal D1R and A2AR is necessary to achieve cognitive improvement in HD mice. Thus we demonstrate that regulation of PKA signaling in the hippocampus is a therapeutically relevant approach in HD.

Disclosures: S.Z. Tyebji: None. **A. Saavedra:** None. **A. Giralt:** None. **J. Alberch:** None. **E. Perez-Navarro:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.09/P9

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: N IH Grant AG039818

Title: Dysregulated behavioral modulation of striatal neuronal processing in the YAC128 mouse model of Huntington's disease

Authors: E. S. ZHANG, S. J. BARTON, *G. V. REBEC;
Program Neurosci & Dept. Psychological & Brain Sci.,, Indiana Univ., Bloomington, IN

Abstract: Transgenic mouse models of Huntington's disease (HD), a dominantly inherited neurodegenerative condition, have been instrumental in identifying the changes in neuronal function that appear to underlie the onset and progression of the behavioral phenotype. R6/2 mice, a widely used model expressing a truncated toxic fragment of the mutant huntingtin gene, develop robust neurological signs early in life and, relative to control mice, show corresponding neuronal changes in several forebrain areas, including the striatum, which shows the first signs of HD neuropathology (Estrada-Sanchez and Rebec, Basal Gang., 2:57-66, 2012). When these mice are symptomatic and behaving freely, striatal neurons are hyperactive and display a firing pattern very different from control (Miller et al., J. Neurophyiol.,100:2205-2216, 2008). Most notable is an R6/2 decrease in striatal burst firing (e.g., fewer bursts/min, a lower percentage of spikes that participate in a burst, and a longer interval between bursts). Because burst activity is a critical component of neural processing, the bursting deficits in the R6/2 striatum likely play an important role in modulating HD behavioral signs. Here, we assessed striatal neuronal activity in behaving YAC128 mice, a full-length transgenic HD model in which the behavioral phenotype develops later than in R6/2s. This later-onset feature allows us to examine striatal activity before and after symptom expression. Like R6/2s, YAC128 mice show many of the same deficits in

striatal bursting, including fewer bursts/min. But when we assessed striatal firing across an age range from pre-symptomatic (10 weeks) to symptomatic (up to 90 weeks), YAC128s differed from control in some but not all aspects of burst firing. For example, some burst parameters changed as the behavioral phenotype developed, while others were present before symptom onset. Thus, although striatal firing changes as symptoms emerge, activity in HD striatum may differ from control even before symptom development.

Disclosures: E.S. Zhang: None. **S.J. Barton:** None. **G.V. Rebec:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.10/P10

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Role of cortical mutant huntingtin in the aberrant behavioral modulation of striatal neuronal activity in the BACHD model of Huntington´s disease

Authors: *A. M. ESTRADA SANCHEZ[1], C. BURROUGHS[1], S. CHEN[1], S. J. BARTON[1], S. CAVALIERE[1], X. W. YANG[2], G. V. REBEC[1];
[1]Program Neurosci & Dept. Psychological & Brain Sci., Indiana Univ., Bloomington, IN;
[2]Psychiatry & Biobehavioral Studies, Geffen Sch. of Med., Univ. of California, Los Angeles, CA

Abstract: Early signs of neuropathology in Huntington's disease (HD), a dominantly inherited neurodegenerative condition, occur in the striatum, which receives major glutamate input from cerebral cortex. In transgenic mouse models of HD, ample evidence indicates a dysregulation of information processing in medium spiny neurons (MSNs), which account for >90% of the striatal neuronal population (Estrada-Sanchez and Rebec, Basal Ganglia, 2:57-66). A critical issue is whether abnormalities in behavioral modulation of striatal signaling can be explained by the presence of the mutant huntingtin gene (mhtt) in MSN neurons, a cell-autonomous process, or by the interaction of cortical and striatal neurons, both of which express mhtt. To address this issue, we used the BAC-Emx1Cre (BE) conditional HD transgenic mouse model, in which mhtt is suppressed in forebrain glutamate projecting neurons, including the cortical pyramidal neurons that project to striatum. We recorded neuronal activity in primary motor cortex and dorsal striatum while the animals behaved spontaneously in an open-field arena and plus maze. Neurobehavioral data also were obtained from BACHD mice, a commonly used transgenic model that expresses full-length mhtt, and wild-type strain controls (FvB/N). Animals ranged in

age between 19 and 60 weeks, which for BACHD mice includes both pre-symptomatic and symptomatic periods. Relative to wild-type, BACHD mice showed characteristic changes in striatal activity similar to those reported for other HD models (Miller et al., J. Neurophysiol. 100:2205-2216), including: changes in burst firing such as decreases in the number of spikes/burst and the percentage of spikes in a burst. These striatal changes were accompanied by characteristic signs of behavioral inflexibility such as increased open-field grooming and decreased turning probability in the plus maze. BE mice, in contrast, showed intermediate changes in burst firing and behavior that were often significantly different from BACHD. Surprisingly, however, cortical neurons in BE mice show increased bursting activity, suggesting possible compensatory effects in response to neuronal inputs containing mhtt. Collectively, our results argue against the cell-autonomous model of HD and support a role for cell interactions in the abnormal firing patterns of striatal MSNs.

Disclosures: A.M. Estrada Sanchez: None. **C. Burroughs:** None. **S. Chen:** None. **S.J. Barton:** None. **S. Cavaliere:** None. **X.W. Yang:** None. **G.V. Rebec:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

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Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH grants NS041669

NIH Grant AG019206

NIH Grant NS045016

Title: Turnover of mutant huntingtin in different neuronal compartments

Authors: *T. ZHAO, Y. HONG, S.-H. LI, X.-J. LI;
Human Genet., Emory Univ., Atlanta, GA

Abstract: Huntington’s disease (HD) is an autosomal dominant, neurodegenerative disease that affects one in every 10,000 Americans. About 200,000 Americans are at risk of inheriting the disease from affected parents. HD patients are characterized by motor, cognitive and neuropsychiatric abnormalities. HD is caused by the expansion of the trinucleotide CAG (>37 units) encoding an expanded stretch of polyglutamine (PolyQ) in the N terminal region of mutant huntingtin (mhtt). Mhtt is neurotoxic and induces neuronal death by disturbing gene expression,

axonal transport, and mitochondrial function. Mhtt is prone to form insoluble aggregates. Appearance of mhtt aggregates is indicative of the accumulation of mhtt. In HD, progressive emergence of mhtt aggregates in neurons is observed. Strikingly, the mhtt aggregates preferentially form in neuronal neurites and nuclei, and few aggregates form in the cytosol of soma. Based on these observations, we hypothesize that the degradation of mhtt is slower in neuronal neurites and nuclei than in cytosol of soma. In order to study degradation rates of mhtt in different neuronal compartments, we conjugate dendra2, a photoconvertible fluorescent protein, to the N-terminal fragmented mhtt (Htt230-130Q) and wild-type htt (Htt230-23Q) that is used as the control. Dendra2 is irreversibly photoconverted from a green to a red fluorescent state with 405nm light in the neuronal compartments. After photoconversion, decline of red signal over time is used to measure the degradation rates of Htt230(130/23Q)-dendra2 in the subcellular compartments. Our results showed that Htt230(130Q)-dendra2 was degraded faster than Htt230(23Q)-dendra2 in the cytosol of soma in primary hippocampal neurons. However, mhtt was stable in the neurites. No difference in degradation rate was seen in neuronal nucleus between mhtt and wild-type htt. Furthermore, turnover of mhtt was faster in the cytosol of soma than in the nucleus and neurites in neurons. Compared to neurons, astrocytes show faster degradation of mhtt than wild-type htt in each compartment. Our data supports the hypothesis that few mhtt aggregates in the cytosol of soma is due to efficient turnover of mhtt in this compartment and that astrocytes are capable of degrading mhtt fast and efficiently to avoid mhtt toxicity.
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Disclosures: T. Zhao: None. **Y. Hong:** None. **S. Li:** None. **X. Li:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.12/P12

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant AG031153

NIH Grant AG019206

Title: Expression of mutant huntingtin in mouse brain astrocytes suppresses BDNF secretion

Authors: *Y. HONG, T. ZHAO, X.-J. LI, S.-H. LI;
Human Genet., Emory Univ., Atlanta, GA

Abstract: Huntington's disease (HD) is an inherited neurological disorder caused by a polyglutamine (polyQ) expansion in the N-terminal region of the protein Huntingtin (htt), and is characterized by the preferential loss of striatal medium-size spiny neurons (MSNs) in the brain. Symptoms usually consist of body weight loss, cognitive deficits, and movement disorders. Previous studies have been mainly focused on the neuronal cells in the brain, as the neuronal cell death in the brains of patients with HD is the most prominent pathology. However, the brain consists of a large number of glial cells, including astrocytes, which support neuronal function and survive. Astrocytes have functions that include promoting neuronal survival, removing toxic materials, and providing neurotrophic factors to neurons. Many studies show that brain-derived neurotrophic factor (BDNF) levels are decreased in the brains of HD patients and animal models, especially in the striatum, leading to the idea that reduced BDNF levels may be responsible for the selective neuronal vulnerability in HD. In this study, we used transgenic mice (Htt-160Q mice) that express N-terminal mutant huntingtin (mhtt) only in astrocytes to study the effect of mhtt on BDNF secretion. Western blotting and q-PCR results show no significant difference of BDNF level in astrocytes between WT and Htt-160Q mice. However, ELISA results demonstrate that the secretion level of mature BDNF decreases in the culture medium of astrocytes from Htt-160Q mice. These results indicate that mhtt may impair secretion of BDNF from astrocytes, which might contribute to the neuronal dysfunction and degeneration in HD. The mechanism of decreased BDNF secretion is under investigation.
Supported by NIA/NIH AG031153 and AG019206

Disclosures: Y. Hong: None. **T. Zhao:** None. **X. Li:** None. **S. Li:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.13/P13

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant AG039818

CHDI Foundation

Title: Altered dopamine release dynamics correlate with behavioral alterations in the YAC128 mouse model of Huntington's disease

Authors: *K. D. BUNNER, A. M. ESTRADA SANCHEZ, G. V. REBEC;
Program in Neurosci. & Dept. Psychological & Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by cognitive and motor deficits. Few studies have examined changes in neurotransmission during the initial hyperkinetic motor period of HD. In part, this is due to the rapid symptom onset seen in certain mouse lines. However, because HD is known to be caused by expansion of the CAG repeat on the huntingtin gene, multiple animal models have been developed. One such model, the YAC128 mouse, has slow disease progression. As is the case with humans, YAC128 mice show striatal and cortical atrophy as well as a hyperkinetic followed by a hypokinetic phenotype. Because dopamine (DA) is known to innervate the striatum as well as play a critical role in movement, striatal DA release and uptake in the YAC128 mice were assessed at 3, 6, 9, and 12 months of age. Using fast scan cyclic voltammetry (FSCV), electrically evoked DA release was monitored in vivo via medial forebrain bundle stimulation. Our results indicate an increase in DA release from 3 to 6 months of age, followed by a decrease in DA during the 9th and 12th month. These results demonstrate changing DA release dynamics, which parallel the biphasic (hyper- and hypo-) phenotype expressed in YAC128 mice. Interestingly, while significantly lower in YAC218 mice compared to wild-type (WT) controls, the rate of DA uptake showed no change throughout the progression of the disorder in the YAC128 mouse. Preliminary immunoblotting data indicates a decrease in the amount of DA transporter present in YAC128 compared to WT control.
Nest building behavior has also been linked to DA and was analyzed throughout disease progression in YAC128 mice. Results revealed YAC128 mice displayed nest building similar to WT controls until 9 months of age, after which YAC128 mice show a progressive decline in nest building activity. These data parallel the decline in DA release during the hypokinetic phenotype. Collectively, our results have important implications for understanding the progression of HD and the mechanisms by which HD pharmacotherapies focused on DA (e.g. tetrabenazine) exert their therapeutic effects.

Disclosures: K.D. Bunner: None. **A.M. Estrada Sanchez:** None. **G.V. Rebec:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.14/P14

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Altered neuronal activity in-vivo in subthalamic nucleus but not globus pallidus in the heterozygous z_Q175 mouse model of Huntington's disease

Authors: H. LIN[1], S. ZHONG[1], S. BENT[1], *H. S. HAIN[2], V. BEAUMONT[3], A. GHAVAMI[1]; [1]Preclinical Testing Group, [2]Behavioral Pharmacol., PsychoGenics, Tarrytown, NY; [3]CHDI Mgmt. / Fndn. Inc, Los Angeles, CA

Abstract:
Huntington's disease (HD) is a lethal autosomal dominant neurodegenerative disease that leads to deficits in motor control and cognitive/psychiatric functions. Chorea, a loss of motor control that is characteristic of HD physical symptoms, is thought to reflect a dysfunction of the indirect pathway (IP) drive arising from a deficit of striatal output to globus pallidus (GP), which in turn innervates subthalamic nucleus (STN). Due to the inhibitory GABAergic projections from GP to STN, it is expected that an increase in GP activity would lead to a decrease in STN neuronal discharge. Electrophysiological studies in basal ganglia slices from symptomatic transgenic BACHD rats carrying full length mutant huntingtin has shown increased GP and decreased STN firing consistent with this hypothesis[1].
We have evaluated spontaneous single unit discharges in GP and STN *in-vivo*, in heterozygous (HET) and homozygous (HOM) z_Q175 mice, a knock-in mouse model of HD which carries either one normal Htt allele and one mutant htt allele with an expanded CAG tract (HET), or two mutant Htt alleles (HOM)[2,3]. Six month-old HET, HOM and WT littermate mice were anesthetized using urethane, and extracellular single unit spikes were recorded in GP or STN. The firing rate of STN neurons appeared to be more sensitive to the presence of mHtt, with lower firing rates in HET and HOM mice compared to WT counterparts; however there was no change in mean firing rate difference found for GP neurons between WT and HET. The lack of rate difference in GP could either stem from less sensitivity of GP neurons to IP dysfunction, or could be due to GP heterogeneity in firing rate and pattern that makes detection of abnormalities in single unit activity harder in GP than STN. Alternatively, an impairment of the hyperdirect pathway
(excitatory glutamatergic cortico-STN projection) could result in a hypoactive STN with minimum impact in GP activity, at least during the early stage of HD neurodegeneration in the z_Q175 mouse model.
1. Zhong et al. (2013). Evaluation of MP-10, a PDE10 inhibitor, on BACHD transgenic rats using dual recording of single units in Globus Pallidus and Subthalamic nucleus. SFN poster 2013.
2. Heikkinen T et al. (2012) PLoS ONE
3. Menalled LB et al. (2012) PLoS ONE
(This study was funded by CHDI Foundation.)

Disclosures: H. Lin: None. **S. zhong:** None. **S. bent:** None. **H.S. Hain:** None. **A. ghavami:** None. **V. Beaumont:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.15/P15

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI A-5303

Title: Characterization of endogenous BDNF isoforms, secretion and signaling in a mouse model of Huntington's disease

Authors: Q. MA, *B. L. HEMPSTEAD;
Weill Cornell Med. Col., NEW YORK, NY

Abstract: Huntington's disease (HD) is a neurodegenerative disease that features striatal cell loss, leading to involuntary movements, cognitive decline, and psychiatric manifestations. Brain-derived neurotrophic factor (BDNF) has been shown to have protective roles for primary striatal neurons expressing mutant huntingtin in vitro, and the primary source of BDNF in the striatum is from anterograde transport from the cortex. A significant decrease of BDNF protein has been reported in the striatum of both HD patients and transgenic animal models. However, the mechanisms underlying the reduction of striatal BDNF and the subsequent selective neuronal atrophy are largely unknown.

To analyze alterations in BDNF levels and downstream signaling cascades in the cortex and striatum of HD knock-in mice, we utilized the zQ175 knock-in mouse model of HD, which has ~190 CAG repeats inserted in human/mouse chimeric exon 1. We demonstrate that mature BDNF levels and TrkB activation are both significantly reduced in the striatum of 9-month-old zQ175 mice, with more modest decreases in the levels in the cortex and the hippocampus. Interestingly, total p75NTR expression is significantly decreased in both the cortex and the striatum of zQ175 mice, but localized induction of p75NTR is observed in the astrocytes within these two brain regions in 12 month old zQ175 mice. Sortilin, a member of Vps10p-domain receptor family that can regulate intracellular trafficking of proBDNF and proBDNF-mediated cell death, is modestly increased. However, SorCS2, another Vps10p family member that interacts with proBDNF, is selectively downregulated in the medium spiny neurons, but its levels are unchanged in the cortex and the hippocampus.

These data suggest that in addition to a decrease of BDNF protein in the striatum, expression of mutant Htt leads to alterations in the level of BDNF receptors, that could contribute to the dysfunction of medium spiny neurons, and the pathogenesis of HD. Ongoing studies are evaluating the processing, trafficking and release in endogenous BDNF, utilizing bdnf-HA knock-in mice to facilitate quantification of BDNF isoforms in the striatum in models of HD.

Disclosures: Q. Ma: None. **B.L. Hempstead:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.16/P16

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: South Dakota Board of Regents

Title: Ubiquilin suppresses accumulation of mutant Huntingtin and motor deficits in HD mice

Authors: C. L. HETTINGER[1], G. DONG[2], Y. LIU[1], L. LU[3], M. J. WATT[1], G. L. FORSTER[1], X. WANG[1], *H. WANG[1];
[1]Univ. of South Dakota Sanford Sch. of Med., Vermillion, SD; [2]Dalhousie Univ., Halifax, NS, Canada; [3]Sun Yat-sen Univ., Guangzhou, China

Abstract:
Huntington's disease (HD) is pathologically characterized by loss of neurons in the striatum and cortex, leading to motor, cognitive and psychiatric impairments, and by the formation of intra-neuronal aggregates in both the cytoplasm and nucleus. Ubiquilin (Ubiquilin 1, Ubqln) is a ubiquitin-like protein and is associated with mutant huntingtin (mHtt) in the aggregates. Our previous studies using cell culture models of HD have indicated that Ubqln facilitates the degradation of mHtt protein and protects cells against mHtt-caused cytotoxicity, suggesting that Ubqln may be a potential therapeutic target for HD. However, this has not been determined in a vertebrate animal model. To further understand the role of Ubqln *in vivo*, we generated Ubqln transgenic (Tg) and conditional knockout (cKO) mice. By crossing Ubqln Tg and cKO animals with HD mice, we generated the HD/Ubqln double transgenic mice and the HD mice deficient for the Ubqln gene, respectively. Compared to the HD single transgenic animals, overexpression of Ubqln in the Ubqln/HD double Tg mice prevents accumulation and aggregation of mutant Htt and attenuates HD motor deficits. In contrast, knockout of Ubqln gene in the HD mouse exacerbates the accumulation of mHtt and HD motor behavior deficits. These observations not only support that enhancement of mHtt degradation is a therapeutic strategy but also indicate that Ubqln is a potential therapeutic target for treating HD.

Disclosures: C.L. Hettinger: None. **G. Dong:** None. **Y. Liu:** None. **L. Lu:** None. **M.J. Watt:** None. **G.L. Forster:** None. **X. Wang:** None. **H. Wang:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

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Program#/Poster#: 242.17/P17

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Utilization of an In-Cell Western™ assay to evaluate putative SIRT1 pathway modulators: PARP inhibition leads to increased SIRT1 activity in an oxidative stress paradigm

Authors: *D. F. FISCHER[1], J. BATE[2], S. DOWLER[2], V. LAZARI[2], S. DIJKSTRA[3], G. MCALLISTER[2], J. WITYAK[4], I. MUNOZ-SANJUAN[4];
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Abstract: Overexpression of SIRT1 is reported to be neuroprotective in a range of preclinical models, including multiple Huntington's disease (HD) mouse models. Therefore, small molecules that are able to modulate SIRT1 activity may lead to neuroprotection in HD. There remains significant controversy over recently identified SIRT1 "activator" molecules, which appear to require fluorescent tag-containing peptide substrates to show SIRT1 "activation." Additionally, there may be less direct, pathway-mediated ways to modulate SIRT1 activity in cells that are not amenable to a biochemical assay using cell-free readouts.
We have therefore developed an In-Cell Western™ assay with the ability to detect modulation of SIRT1 activity in a cellular context, focusing on acetylation of the well-characterized SIRT1 substrate, p53K382. Assay development experiments have shown the ability of this assay to detect both pharmacological inhibition and overexpression of SIRT1. Experiments performed using this system reveal a significant increase of p53 acetylation upon acute challenge with oxidative stress. Further experiments reveal that the DNA damage response caused by oxidative stress results in a rapid depletion of intracellular NAD+ and ATP, consistent with activation of the PARP (poly ADP-ribose polymerase) resulting in a reduction of NAD+ dependent SIRT1 activity and subsequent increase in p53 acetylation. Treatment of cells with PARP inhibitors, such as olaparib, leads to protection from the NAD+/ATP depletion caused by oxidative stress and may represent alternative methods of activating the SIRT1 pathway leading to neuroprotection. The significance of this pathway in a Huntington's disease context is being investigated.

Disclosures: D.F. Fischer: A. Employment/Salary (full or part-time):; BioFocus. **J. Bate:** A. Employment/Salary (full or part-time):; BioFocus. **S. Dowler:** A. Employment/Salary (full or part-time):; BioFocus. **V. Lazari:** A. Employment/Salary (full or part-time):; BioFocus. **S. Dijkstra:** A. Employment/Salary (full or part-time):; Galapagos. **G. McAllister:** A. Employment/Salary (full or part-time):; BioFocus. **J. Wityak:** A. Employment/Salary (full or

part-time):; CHDI Management/CHDI Foundation. **I. Munoz-Sanjuan:** A. Employment/Salary (full or part-time):; CHDI Management/CHDI Foundation.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.18/P18

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: To fold or not to fold? Novel methods for identification of modifiers of mutant huntingtin conformation

Authors: *R. KUHN[1,2], A. WEISS[2], M. VERANI[2], L. AZZOLINI[2], V. FODALE[2], H. PARK[3], A. CARICASOLE[2];
[1]Redbark Gmbh, Riehen, Switzerland; [2]IRBM PROMIDIS, Pomezia, Italy; [3]CHDI Fndn., Los Angeles, CA

Abstract: Huntington's disease is caused by a CAG triplet repeat expansion in exon 1 of the huntingtin gene, encoding an abnormal expanded polyglutamine (polyQ) tract. The expanded polyQ alters protein folding and leads to aggregation and accumulation in the nucleus and cytoplasm with consequent disturbance of many cellular processes, which ultimately result in neuronal cell death. Recent evidence highlights a potential role of post-translational modifications (PTMs) in the N-terminal 17 amino acids (N17) and neighboring regions of the polyQ domain. Also, X-ray crystallography and studies with conformation-specific antibodies indicated that N17 sequences adopt multiple conformations and strongly influence aggregation and toxicity properties of mutant huntingtin.
Our studies with huntingtin-specific TR-FRET assays (sensitive for conformational changes) also suggest that wild type and mutant huntingtin can adopt different conformations when the purified proteins or cell lysates are incubated at different temperatures prior to TR-FRET assays. Specifically, we observed that temperature changes in huntingtin FRET signals are strongly and progressively modulated by the polyQ length of the protein, i.e. the huntingtin TR-FRET signal is low with long polyQ and high at short polyQ length suggesting that increasing polyQ length restricts the conformational flexibility of the huntingtin protein. This observation was reproduced with purified huntingtin fragments, cell lysates of huntingtin fragments and full-length huntingtin. Interestingly, the conformational flexibility of the mutant protein is strongly reduced above polyQ lengths of >35-40, namely the threshold above which the disease invariably

develops. Clearly, these assays represent potentially valuable tools, and require further studies to validate their relevance to other mutant huntingtin properties and to different translationally and clinically relevant biological matrices, such as tissues from animal models and human HD samples.

Disclosures: R. Kuhn: A. Employment/Salary (full or part-time):; IRBM PROMIDIS. 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.; CHDI Foundation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRBM PROMIDIS. **A. Weiss:** A. Employment/Salary (full or part-time):; IRBM PROMIDIS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRBM PROMIDIS. **M. Verani:** A. Employment/Salary (full or part-time):; IRBM PROMIDIS. **L. Azzolini:** A. Employment/Salary (full or part-time):; IRBM PROMIDIS. **A. Caricasole:** A. Employment/Salary (full or part-time):; IRBM PROMIDIS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRBM PROMIDIS. **V. Fodale:** A. Employment/Salary (full or part-time):; IRBM PROMIDIS. **H. Park:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.19/Q1

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NRF grant 2012-003338

NRF grant 2012-0009221

NRF grant 2011-0030928

Title: The effects of resveratrol on mitochondrial function mechanism in Huntington’s disease model mice

Authors: *J. JEON, H. SEO;
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Abstract: Huntington's disease (HD) is a neurodegenerative disease, which leads to progressive disability with both psychiatric and cognitive impairment. HD is caused by a cytosine-adenine-guanine (CAG) repeat expansion in the huntingtin gene. The mutant huntingtin protein induces neuronal dysfunction and eventual cell death. It was reported that mitochondrial dysfunction and morphological dysregulation in HD impacted on neuronal cell death. In this study, we administrated resveratrol (RSV) that functions as antioxidant and sirtuin1 (Sirt1) activator to HD model mice of YAC128. We determined the epigenetic controls of mitochondrial function in HD. In addition, we checked the changes of progressive pathology in HD mice brain. We detected that the function of mitochondria was improved by RSV through ATP elevation and attenuation of cellular oxidative stress levels. In addition, we detected the improved motor ability by RSV administration in HD mice model. These results suggest that the mitochondrial regulation can control not only mitochondrial function, but also HD motor impairment.

Disclosures: J. Jeon: None. **H. Seo:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.20/Q2

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Neuronal autophagy in Huntington’s disease

Authors: *A. WEISS[1], R. BOGGIO[1], V. FODALE[2], A. CARICASOLE[2], D. MARCHIONINI[3], R. KUHN[2];
[1]PROMIDIS, [2]IRBM PROMIDIS, Pomezia, Italy; [3]CHDI, Los Angeles, CA

Abstract: Autophagy is an essential degradation pathway conserved in all eukaryotes that controls cellular homeostasis by eliminating protein aggregates and damaged organelles. Induction of macroautophagy through pharmacological or genetic means has been shown to to enhance clearance of aggregated huntingtin and to reduce toxicity of huntingtin in cells. However, knowledge of neuronal macroautophagic process is still largely inferred upon findings in non-neuronal contexts.
We have developed quantitative, specific and sensitive immunoassays that are independent of reporter constructs to interrogate macroautophagy at multiple levels in cells,neurons and in vivo model-derived material to characterize the autophagic process in disease-relevant Huntington’s disease models and to identify novel neuronal autophagy inducers. We present novel

immunoassays that are sufficiently robust and scalable for high throughput screening and applicable to monitoring autophagic activities in preclinical and clinical HD samples.

Disclosures: **A. Weiss:** A. Employment/Salary (full or part-time):; IRBM PROMIDIS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRBM PROMIDIS. **R. Boggio:** A. Employment/Salary (full or part-time):; IRBM PROMIDIS. **V. Fodale:** A. Employment/Salary (full or part-time):; IRBM PROMIDIS. **A. Caricasole:** A. Employment/Salary (full or part-time):; IRBM PROMIDIS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRBM PROMIDIS. **D. Marchionini:** A. Employment/Salary (full or part-time):; CHDI. **R. Kuhn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IRBM PROMIDIS.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.21/Q3

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant NS066339

Title: Impairment of parkin-mediated mitophagy in Huntington’s Disease

Authors: M. SACINO, N. LAMBRINO, C. ERIE, M. LU, *J. WEI;
Florida Atlantic Univ., BOCA RATON, FL

Abstract: Mitochondrial dysfunction has emerged as a key pathogenic mechanism in the progression of Huntington’s Disease (HD). Damaged mitochondria are accumulated in HD, suggesting impairment in the removal of dysfunctional mitochondria in these cells. The interaction between mitophagy, the macro-autophagic removal of damaged mitochondria, and mitochondrial dynamics has been linked to the removal of dysfunctional mitochondria. Strong evidence supports the role of parkin in mediating mitophagy. In this study, we sought to investigate the functional impairment of the Parkin-mediated mitophagy in two HD cell lines. We overexpressed EYFP-parkin in striatal cell line (STHdhQ7 and STHdhQ111), or a PC12 huntingtin-inducible cell line (htt23Q and htt145Q). Using Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) to depolarize mitochondria, we showed that parkin translocated to depolarized mitochondria and ubiquintinated mitochondria proteins in both cells expressing wild type or mutant huntingtin (mhtt) by immunofluorescence. However, we noticed that

damaged mitochondria formed larger perinuclear aggregates in mhtt cells while they appeared smaller and isolated in normal cells. We reasoned that cells expressing mhtt exhibited decreased levels of mitochondrial clearance compared to wild type cells because of the formation of larger dysfunctional mitochondrial aggregates. Consistently, we showed that mitochondrial clearance was decreased by ~50% in Hela cells overexpressing N-terminal htt103Q compared to Hela cells with N-terminal htt23Q after a 24-hour CCCP treatment. Immunoblotting assay and immunofluorescence data confirmed disregulated proteasomal degredation of Mitofusin 1 and 2, outer mitochondrial membrane proteins essential to mitochondrial fusion, in PC12 htt145Q cells. Our data suggest impairment in the canonical pathway of linking the Ubiquitin-Proteasome System (UPS) activation with mitophagy in HD cells. Subsequently, depolarized mitochondria may not be able to enter autophagosomes for lysosomal fusion and degradative clearance.

Disclosures: M. Sacino: None. **N. Lambrino:** None. **C. Erie:** None. **M. Lu:** None. **J. Wei:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.22/Q4

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Cure Huntington Disease Initiative

Canadian Institutes of Health Research

Michael Smith Foundation for Health Research

Title: Bidirectional control of PSD-95 clustering by non-pathogenic huntingtin

Authors: *M. P. PARSONS, R. KANG, L. A. RAYMOND;
Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Decreasing expression of the huntingtin protein (htt) is a promising strategy for the treatment of the neurodegenerative disorder Huntington disease (HD). In HD, the presence of mutant huntingtin (mhtt) results in synaptic alterations and subsequent cell death. Particularly vulnerable are the striatal spiny projection neurons (SPNs), whose dysfunction and eventual degeneration result in severe motor symptoms that are the hallmark of HD. Unfortunately, many of these htt-lowering strategies are not necessarily specific for mhtt over htt and the lowering of the non-pathogenic protein could be detrimental to cellular function. However, the role of htt in

synaptic communication is unknown. Here, we used immunohistochemical analyses and electrophysiological measurements in co-cultures of striatum and cortex to show that htt plays an important role in protein organization in the SPN postsynaptic density. Specifically, we found that htt overexpression resulted in an increase in the palmitoylation and synaptic clustering of the integral scaffolding protein PSD-95. Surprisingly, this effect was dependent on presynaptic, not postsynaptic htt overexpression and was mimicked and occluded by the addition of the trophic factor BDNF to the culture media. Despite the enhanced clustering, we saw no associated increase in the amount or composition of NMDA or AMPA glutamate receptors at cortico-striatal synapses. Conversely, htt knockdown decreased the size of PSD-95 clusters and preliminary electrophysiological data suggests an associated reduction in AMPA receptor currents at these synapses. In all, our results demonstrate that wild-type htt can influence the organization of synaptic proteins and cautions the use of non-specific htt-lowering strategies for the treatment of HD.

Disclosures: M.P. Parsons: None. **R. Kang:** None. **L.A. Raymond:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.23/Q5

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Grant RevNo002

Title: Characterization of autophagy in a Huntington’s disease mouse model

Authors: *J. LIM, Z. YUE;
Biol., Mount Sinai Sch. of Med., New York, NY

Abstract: Huntington's disease (HD) is a devastating neurodegenerative movement disorder characterized as one of the polyglutamine (polyQ) disease family. Huntingtin (Htt) protein with polyQ expansion leads to accelerated protein aggregation that is causal to the disease. Emerging evidence has shown that mutant Htt can be degraded by autophagy, which is a catabolic pathway responsible for the turnover of long-lived proteins, damaged organelles and large protein aggregates that cannot be degraded by the ubiquitin proteasome system. Therefore enhanced autophagic degradation of polyQ-expanded Htt protein is expected to be beneficial, while impairment of its degradation would accelerate the disease progression. Other evidence suggests that mutant Htt impairs autophagy in an HD cell model. It remains to be examined, however, whether autophagy is altered in the CNS of the HD animal model. In this study we characterized

autophagic activity using a knock-in mouse model expressing mutant Htt with an 175 glutamine repeat (Q175). First we examined the expression levels of numerous autophagy-related proteins in the cortex and striatum including LC3, p62, Vps34, Atg14L, p-mTOR, p-ULK1 and Beclin 1 at multiple ages of Q175 mice. Second, we crossed transgenic GFP-LC3 reporter mice to Q175 HD mice and analyzed autophagic activity by monitoring GFP-LC3 distribution in the striatum. Furthermore, we carried out lipid kinase assays monitoring the Atg14L-associated Vps34 complex, which is a class III phosphatidylinositol-3 kinase involved in the regulation of autophagy induction, in HD brain lysates. We will report our findings from the above studies and discuss the implication of the results in the development of autophagy-modulating drugs to treat HD.

Disclosures: J. Lim: None. **Z. Yue:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.24/Q6

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Canadian Institutes of Health Research

Cure Huntington Disease Initiative

Michael Smith Foundation for Health Research

Title: Region-specific effects of huntingtin overexpression on the palmitoylation and clustering of PSD-95

Authors: *C. BUREN, M. P. PARSONS, R. KANG, L. WANG, L. A. RAYMOND; Psychiatry, The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Huntington disease (HD) is caused by a polyglutamine (polyQ) expansion in the N-terminal region of the huntingtin protein (Htt). This mutation results in synaptic dysfunction and eventual neurodegeneration that particularly influences the spiny projection neurons (SPNs) of the striatum. While it is clear that a toxic gain-of-function of mutant Htt contributes to disease pathogenesis, many lines of research suggest that a loss of wildtype Htt function may also play a significant role in HD progression. Despite this, we know little regarding the role of Htt in synaptic function. By comparing cortico-striatal co-cultures from FVB/N wildtype (WT) versus YAC18 mice, which overexpress non-pathological human Htt, we have found that Htt

overexpression increases synaptic clustering of the important scaffolding protein PSD-95, thereby suggesting a role for Htt in synaptic function. Importantly, this effect was observed in SPN dendrites receiving excitatory input from cortical neurons. Here, we examined PSD-95 palmitoylation - a post-translational modification that promotes PSD-95 clustering - in different brain regions from WT and YAC18 mice. Consistent with enhanced PSD-95 clustering, we observed an increase in PSD-95 palmitoylation in striatal tissue from YAC18 mice compared to WT mice. Interestingly, this effect was not a global phenomenon as we found no evidence for enhanced PSD-95 palmitoylation in cortical tissue, nor any change in PSD-95 clustering in the dendrites of YAC18 cultured cortical neurons. As in the striatum, increased palmitoylation of PSD-95 was also observed in YAC18 hippocampal tissue. Studies of PSD-95 cluster size in cultured hippocampal neurons are ongoing and will be presented at the meeting. Finally, we have previously published that YAC18 striatal neurons are protected from NMDA-induced apoptosis, compared with WT, and we have recently found an increase in basal levels of nuclear phospho-CREB in co-cultured YAC18 SPNs. Experiments to determine whether Htt overexpression protects cortical or hippocampal neurons from NMDAR-mediated death, or alters basal levels of nuclear pCREB are underway. Together, our data suggest that huntingtin can influence synaptic protein localization in a region-specific manner.

Disclosures: C. Buren: None. **M.P. Parsons:** None. **R. Kang:** None. **L. Wang:** None. **L.A. Raymond:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.25/Q7

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Hatch WYO-438-09

NIH 5P20RR015640

Title: Differential effects of a neuroprotective selenium dose on selenoprotein transcripts levels in brains of wild-type and transgenic Huntington’s disease mice

Authors: *Z. LU[1,2], J. CHEN[1,2], E. MARKS[1,2], J. MOLINE[1], M. RAISBECK[1], L. F. BARROWS[1], I. VOLITAKIS[3], A. BUSH[3], J. H. FOX[1,2];
[1]Dept. of Vet. Sci., [2]Interdepartmental Grad. Neurosci. Program, Univ. of Wyoming, Laramie, WY; [3]Mental Hlth. Res. Institute, Univ. of Melbourne, Parkville, Australia

Abstract: Huntington's disease (HD) is a progressive neurologic disorder caused by polyglutamine-expanded mutant huntingtin protein (mhtt) which misfolds and accumulates in neurons resulting in selective brain degeneration. Selenium is an essential nutrient that is required for normal brain function. Defects of brain selenium metabolism can induce neurodegeneration. We have found that selenium supplementation provides neuroprotection in N171-82Q HD mice, including improved motor endurance on Rota-rod, increased brain mass and decreased amounts of mutant huntingtin aggregates. Understanding the mechanism(s) of protection by selenium supplementation is important as it may provide avenues for treatment optimization and reveal peripheral treatment biomarkers. The function of selenium in brain is mainly mediated by selenoproteins, therefore we are investigating whether selenium supplementation increases expression of selenoprotein-encoding genes in the brains of N171-82Q HD mice. Female mhtt (+/-) and mhtt (-/-) mice were provided with selenite in drinking water and / or control water, from 6-14 weeks of age. All 24 selenoprotein transcripts are being analyzed by quantitative real-time PCR. Results show that selenium supplemented N171-82Q mice have a significant increase in brain glutathione peroxidase 3 (GPX3) mRNA. Further, for several of the genes studied (e.g. thioredoxin reductase 1, 2 and 3, selenoprotein S) the effect of selenium supplementation is genotype dependent (significant genotype x treatment interaction effect). Our findings show differential transcriptional responses to selenium supplementation between wild-type and HD mice and suggest that some selenoprotein-encoding genes could be mediating the protective effect of selenium supplementation in HD mice.

Disclosures: Z. Lu: None. **J. Chen:** None. **E. Marks:** None. **J. Moline:** None. **M. Raisbeck:** None. **L.F. Barrows:** None. **I. Volitakis:** None. **A. Bush:** None. **J.H. Fox:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

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Program#/Poster#: 242.26/Q8

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: This work was supported by grants to S.H.S. from the US Public Health Service grant (MH18501)

This work was supported by grants to S.H.S. from CHDI

Title: Golgi protein ACBD3 mediates neurotoxicity associated with Huntington's Disease

Authors: *J. I. SBODIO, B. D. PAUL, C. E. MACHAMER, S. H. SNYDER;
Johns Hopkins Univ., Baltimore, MD

Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative condition characterized by emotional disturbances, motor dysfunction and neuronal loss. Mutations in the gene encoding the protein huntingtin results in an expansion of CAG codon generating an extension of glutamines responsible for misfolding and aggregation. Mutant huntingtin (mHtt) is expressed throughout the brain and the peripheral tissues, however, HD patients show a predominant degeneration of the corpus striatum and lesser damage to the cortex. The striatal association of the disease can be explained by the binding of the striatal-specific protein Rhes (Ras Homolog Enriched in Striatum) to mHtt, which enhances mHtt toxicity. Here we report that the Golgi protein ACBD3 (Acyl-CoA binding Domain Containing 3) interacts with Rhes and mutant Huntingtin to mediate toxicity. We observe elevated levels of ACBD3 in the striatum of HD patients, in a neuronal cell line with extended glutamine repeats, as well as in the brain of HD mouse models. ACBD3 levels are upregulated by Golgi stress, ER stress and mitochondrial stress, suggesting that the stress induced by mHtt can elicit neurotoxicity through ACBD3-Rhes-mHtt.

Disclosures: J.I. Sbodio: None. **B.D. Paul:** None. **C.E. Machamer:** None. **S.H. Snyder:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.27/Q9

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH P50: GM085764

NS060847

Title: Altered Rpt6 proteasome function in S. cerevisiae

Authors: *E. M. MARQUEZ-LONA;
Biol. Sci., UCSD, La Jolla, CA

Abstract: The inherent turnover rate (half-life, t ½) for any given protein is determined by a combination of its synthesis and degradation. Importantly, cell biological signals are capable of altering rates of synthesis, degradation or both. Protein degradation via the ubiquitin proteasome system has proven to play a fundamental role in the central nervous system for the development, maintenance and remodeling of synaptic connections. Examining the mechanisms underlying synaptic plasticity, which alter the signaling dynamics of a synapse, we have recently found that the 26S proteasome, is itself regulate in a dynamic fashion (Djakovic et al., 2009). We have also

identified a specific phosphorylation event on the 19S ATPase subunit, Rpt6, which controls the activity and distribution of proteasomes in neurons (Djakovic-MarquezLona, 2012). Since UPS dysfunction is attributed to the pathogenesis of several neurodegenerative diseases (e.g. Alzheimer Disease, Huntington Disease, and Parkinson Disease), using a yeast model of altered Rpt6 phosphorylation (S120A, S120D) and proteasome function we have evaluated the functional relevance of proteasome phosphorylation in various stress-response and disease-related pathways. We find that mutations preventing Rpt6 phosphorylation, are susceptible to proteotoxic stress and protein aggregate formation.

Disclosures: E.M. Marquez-Lona: None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.28/Q10

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NS036812-15

Title: L-12/15 lipoxygenase (L-12/15 LO) null mutant mice show enhanced motor behavioral deficits and increased moribundity in response to 3-nitroproprionic (3-NP) acid treatment

Authors: *R. C. AKUMUO, Y. YANG, Y. HE, S. J. HEWETT;
Biol., Syracuse Univ., Syracuse, NY

Abstract: Oxidative stress results from an imbalance in the production and/or removal of reactive oxygen species resulting in DNA, protein and lipid peroxidation. Recent studies demonstrate an increase in L-12/15 LO immunoreactivity in pathologically affected Alzheimer's disease (AD) brains, that correlated with the extent of lipid peroxidation, suggesting that increased L-12/15 LO activity may contribute to the pathogenesis of AD (Pratico et al 2004, *Am J Pathol* 164:1655-62; Yao et al 2005, *Ann Neurol* 58:623-6). Additionally, mice null for the L-12/15 LO isoform have been reported to be less susceptible to cerebral ischemic damage, an acute neurological injury also associated with oxidative stress (Khanna et al 2005, S*troke* 36:e144-52; van Leyen et al 2006, *Stroke* 37:3014-8). In keeping with these *in vivo* results, we and others determined that when L-12/15 LO activity is disrupted, either genetically or pharmacologically, neurons are less susceptible to oxytosis ─ a non-receptor-mediated glutamate-induced oxidative cell death. Herein, we compared the behavioral motor function between L-12/15 LO null mutant mice (JAX: Strain B6.129S2-Alox15^{tm1Fun}/J) and wild-type littermate control mice that followed systemic injection of 3-NP, a succinate

dehydrogenase inhibitor that produces energy depletion, oxidative stress and striatal lesions. To induce injury, male L-12/15 LO null mutant animals (n = 18) and male wild-type littermate control mice (n = 15) received two i.p. daily injections (8-12 h apart) as follows: 20mg/kg x 2d, 30mg/kg x 2d, 50mg/kg x 1d and 60mg/kg x 4d (total cumulative dose = 780 mg/kg in 9 days). A three level scale assessed the severity of the following five items (maximal score=10): hindlimb clasping, reduced locomotor activity, hindlimb dystonia, truncal dystonia and balance adjustments to a postural challenge. Mice were rated before each injection during the intoxication procedure. Surprisingly, we found that severity of the motor system deficits at days 7 and 9 following systemic 3-NP in the L-12/15 LO knockout cohort was greater than in wild-type littermate controls. Additionally, nearly twice the number of the L-12/15 LO null mutant mice (9/18) became moribund when compared to their wild-type littermate control mice (4/15), necessitating sacrifice. This could not be explained by differential metabolism of 3-NP between the different groups as striatal succinate dehydrogenase activity was inhibited by a single acute dose (200mg/kg) to the same extent in both genotypes. Overall, we conclude that animals null for the L-12/15 LO gene are more susceptible to the toxic effects of 3-NP treatment.

Disclosures: R.C. Akumuo: None. **Y. Yang:** None. **Y. He:** None. **S.J. Hewett:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.29/Q11

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

NINDS

The NY State Stem Cell Program (NYSTEM)

Title: Mutant huntingtin transcriptionally represses the cholesterol biosynthetic pathway in striatal astrocytes

Authors: *A. BENRAISS, A. CORNWELL, D. CHANDLER-MILITELLO, M. J. TONER, S. WANG, S. A. GOLDMAN;
Ctr. for Translational Neuromedicine and Dept. of Neurol., Univ. of Rochester Med. Ctr., ROCHESTER, NY

Abstract: Huntington's disease (HD) is caused by an expansion of the CAG trinucleotide repeat of the first exon of the gene encoding huntingtin (Htt). Although striatal neuronal death is a hallmark of HD, the extent to which neuronal death is cell-autonomous or is instead dependent upon paracrine interactions with affected astrocytes has been unclear. To address the specific role of astrocytic pathology in the pathogenesis of HD, we investigated mutant Htt-associated changes in gene expression by striatal astrocytes. To this end, we studied astrocytes acutely isolated from the R6/2 mouse model of HD, using Glt1-based fluorescence activated cell sorting (FACS) followed by mRNA expression profiling by Affymetrix Mouse-430 2.0 microarrays. In particular, striatal tissue from 12 week-old R6/2 mice (150 CAG; n = 6; 5-9 mice/sort) and their wild-type littermate controls (n = 5; 4-9 mice/sort) was dissected and sorted by 2-color FACS using antibodies directed against the astrocytic glutamate transporter 1 (Glt1) and the microglial ectodomain CD11b, so as to isolate microglia-free striatal astrocytes. Analogous sorts were obtained from wild-type age- and strain-matched control mice as well. Differential gene expression analysis revealed profound mHtt-associated changes in the gene expression patterns of both astrocytes and microglia. Among Glt1-defined astrocytes, 619 transcripts were up-regulated and 591 were suppressed as a function of mutant Htt expression ($p<0.01$). In particular, functional enrichment analysis on Gene Ontology terms revealed the sharp down-regulation of transcripts encoding essentially every single transcript within the cholesterol biosynthetic pathway, including: Cyp51a1; Dhcr7; Dhcr24; Fdft1; Hmgcr; Hmgcs2; Lss; Msmo1; Mvd; Mvk; Nsdhl; Sc5dl; Sqle; Tm7sf2 (FDR cut-off $p<0.01$). These data suggest the mHtt-dependent perturbation in astrocytes of a transcriptional regulator common to the entire family of cholesterol pathway genes. Together, these results highlight the potential contribution of glial dysfunction to HD pathology, and suggest that the coordinated inhibition of cholesterol synthesis may be important to HD pathogenesis, while its targeted reversal may have therapeutic potential.

Disclosures: A. Benraiss: None. **A. Cornwell:** None. **D. Chandler-Militello:** None. **M.J. Toner:** None. **S. Wang:** None. **S.A. Goldman:** None.

Poster

242. **Huntington's Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 242.30/Q12

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Association Huntington France

Huntington Society of Canada

Title: Role of vesicular glycolysis in fast axonal transport

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Abstract: Impairments in fast axonal transport (FAT) are a common feature in many neurodegenerative diseases, highlighting the importance of transport in neurons. FAT is an active process that occurs via the consumption of ATP by molecular motors. We have previously shown that FAT does not depend on mitochondrial ATP, but on glycolytic ATP produced on vesicles, due to the presence on vesicles of the glycolytic enzyme GAPDH.
Here we demonstrate that all of the components of the glycolytic machinery are specifically associated to motile vesicles. These enzymes were detected by mass spectrometry analysis of purified motile vesicles and further validated by immunoblotting. Indeed, immunofluorescent staining in cortical neurons, show that glycolytic enzymes colocalise with BDNF containing neurons. Using live imagining of BDNF-mCherry vesicles in cortical neurons grown in microfluidic chambers, we were able to show that Pyruvate Kinase (PK), one of the two ATP producing glycolytic enzymes, is essential for axonal transport. Knockdown of this enzyme reduces the velocity of BDNF-mCherry vesicles. Furthermore, addition of PK's substrate, rescues the transport deficit caused by the knocking down of GAPDH, which is upstream PK. This suggests that all of these enzymes are needed on vesicles to efficiently sustain these chained reactions that generate ATP locally to support FAT.
This study on the energetic independence of transport could give a new insight into the mechanisms regulating axonal transport and have an impact in the study of neurodegenerative diseases.

Disclosures: M.V. Hinckelmann: None. **D. Zala:** None. **C. Niehage:** None. **B. Hoflack:** None. **F. Saudou:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.01/Q13

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS27036

INSERM

Title: C1q induction and activation of the complement pathway do not contribute to ALS toxicity in mutant SOD1 mice

Authors: *C. S. LOBSIGER[1,2], S. BOILLEE[1,2], C. D. POZNIAK[3], A. M. KHAN[4,2], M. MCALONIS-DOWNES[2], J. W. LEWCOCK[3], D. W. CLEVELAND[2];
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Abstract: Accumulating evidence from mice expressing ALS-causing mutations in SOD1 has implicated pathological immune responses in motor neuron degeneration. This includes microglial activation, lymphocyte infiltration and induction of C1q the initiating component of the classic complement pathway that is part of the protein-based arm of the innate immune response. Our previous studies using laser-microdissection based gene expression profiling have identified C1q induction in motor neurons of different ALS mouse models expressing dismutase active or inactive SOD1 mutants. Robust induction early in the ALS disease course is now identified for multiple complement components (including C1q, C4 and C3) in spinal cords of SOD1 mutant expressing mice. These findings are consistent with initial intraneuronal C1q induction, followed by global activation of both classic C1q- and alternative C3-dependent complement pathways, potentially adding to an ongoing deleterious neuroinflammatory response in ALS. We now tested if global complement activation are mechanistic contributors to ALS disease by genetic deletion of C1q and C3 in SOD1 mutant ALS mice. Elimination of C1q induction and classic complement cascade activation that follows from it produced changes in microglial morphology and synaptic densities during disease. Nevertheless and in contrast to expectations, in C1q-deleted ALS mice global onset and progression of disease are unaffected and survival is unchanged in both C1q and C3-deleted ALS mice. These results establish that C1q induction and global complement activation do not contribute significantly to SOD1 mutant-mediated ALS pathogenesis in mice.

Disclosures: C.S. Lobsiger: None. **S. Boillee:** None. **C.D. Pozniak:** None. **A.M. Khan:** None. **M. McAlonis-Downes:** None. **J.W. Lewcock:** A. Employment/Salary (full or part-time):; Genentech Inc, South San Francisco, CA 94080. **D.W. Cleveland:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

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Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: FONDECYT 1120512

FONDEF D10I1077

CARE-Chile-UC (PFB 12/2007)

A. Martinez acknowledges to CONICYT Chile

Title: The c-Abl tyrosine kinase is activated in amyotrophic lateral sclerosis motor neurons and participates in transgenic SOD1(G86R) mice neurodegeneration

Authors: *A. MARTINEZ[1,2], C. VALLS[1,2], C. HETZ[3], A. R. ALVAREZ[1,2];
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Abstract: The c-Abl tyrosine kinase is a ubiquitous protein that participates in the response to cellular stress, regulating apoptosis in neurons. c-Abl has been implicated in neurodegenerative disorders such as Alzheimer, Parkinson and Niemann Pick diseases. The Amyotrophic Lateral Sclerosis (ALS) is a lethal neurodegenerative disease in which the oxidative and endoplasmic reticulum (ER) stress are associated to the loss of motor neurons. Accumulation of mutant forms of the superoxide dismutase 1 (SOD1) has been proposed as one of the causes of this disease; however, the molecular mechanisms involved in the motor neuron loss are not completely understood. We hypothesized that c-Abl is activated in the SOD1 model and it participates in motor neurons neurodegeneration.
To analyze the role of c-Abl in ALS, we first evaluated the activation of c-Abl (through its Tyr412 phosphorylation) under experimental ER stress, by tunicamycin or thapsigargin treatment of primary cultured cells derived from rat spinal cord. We found that c-Abl was early activated under ER stress induction in spinal cord-cultured cells. Also, overexpression of mutant SOD1 in NSC34 cells, a motor neuron-like cell line, induced c-Abl activation. Concordantly to our *in vitro* data, we found increased levels of activated c-Abl in symptomatic transgenic ALS mice, SOD1(G86R), what was specifically localized in motor neurons of the spinal cord. Moreover, the treatment of ALS mice with the FDA-approved inhibitor of c-Abl Imatinib, delayed the paralysis and weight loss. Furthermore, ALS mice exposed to Imatinib treatment exhibited a significant increased survival rate.
Our results suggest a role of c-Abl tyrosine kinase in ALS associated neurodegeneration. Thus, c-Abl inhibition could be a therapeutic target to ameliorate the disease progression.

Disclosures: A. Martinez: None. **C. Valls:** None. **C. Hetz:** None. **A.R. Alvarez:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.03/Q15

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Grant from FVG regional government

Title: Ischemia-like conditions *In vitro* damage rat spinal cord via complex cell death mechanisms

Authors: E. BIANCHETTI[1], M. MLADINIC[2], *A. NISTRI[1];
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Abstract: New spinal cord injury (SCI) cases are frequently due to non-traumatic causes, including vascular disorders. To develop mechanism-based neuroprotective strategies for acute SCI requires full understanding of the early pathophysiological changes to prevent disability and paralysis. The immediate damage spreads from the initial site through excitotoxicity and metabolic dysfunction (ischemia, free radicals and neuroinflammation) to surrounding tissue (secondary damage). The aim of our study was identifying molecular and cellular mechanisms underlying this process and triggered by a pathological medium (PM) mimicking ischemia in the neonatal rat spinal cord in vitro. We previously showed that extracellular Mg2+ (1 mM) worsened PM-induced damage and inhibited locomotor function. The current study sought to identify the cells affected by PM with Mg2+, and the associated molecular death pathways. Our focus was on the spinal lumbar region which contains the locomotor networks for the hindlimbs. The present study indicated that 1 h PM+Mg2+ application induced delayed pyknosis chiefly in the spinal white matter via overactivation of poly (ADP-ribose) polymerase 1 (PARP1), suggesting cell death mediated by the process of parthanatos that was largely suppressed by pharmacological block of PARP-1, and also via caspase 3-dependent apoptosis. Grey matter damage was less intense and concentrated in dorsal horn neurons and motoneurons that became immunoreactive for the mitochondrial apoptosis-inducing factor (the intracellular effector of parthanatos) translocated into the nucleus to induce chromatin condensation and DNA fragmentation. Immunoreactivity to TRPM ion channels believed to be involved in ischemic brain damage was also investigated. TRPM2 channel expression was enhanced 24 h later in dorsal horn and motoneurons, while TRPM7 channel expression concomitantly decreased. Conversely, TRPM7 expression grew earlier (3 h) in white matter cells, while TRPM2 remained undetectable. Our results show that extracellular Mg2+ amplified the consequences of dysmetabolic SCI to comprise not only white matter parthanatos and apoptosis, but also motoneuronal degeneration via PARP1-dependent pathways with distinct changes in their TRPM expression. Supported by a grant from FVG regional government.

Disclosures: E. Bianchetti: None. **A. Nistri:** None. **M. Mladinic:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.04/Q16

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Hans and Ilse Breuer Stiftung Doctoral Research Scholarship

Title: TDP-43 and translational control in neurodegenerative disorders

Authors: *K. MILLER[1], K. DUNCAN[2];
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Abstract: TDP-43 is an RNA-binding protein (RBP) implicated in etiology of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), the most common motor neuron disease, and frontotemporal lobar degeneration (FTLD). In diseased cells, TDP-43 frequently redistributes from the nucleus to the cytoplasm, where it has been found to form inclusions. Additionally, numerous TDP-43 patient mutations have been identified. Model organism studies have shown that misregulated TDP-43 causes motor deficits and motor neuron degeneration, and that TDP-43's RNA-binding ability is necessary to incur these defects. Transcriptome-wide RNA interaction studies revealed that TDP-43 binds to thousands of mRNAs and regulates splicing of a large subset of these. Nevertheless, how TDP-43 contributes to disease remains unclear. TDP-43's relocalization from the nucleus to the cytoplasm in disease-affected neurons raises a fundamental, unanswered question: is disease due to a loss of nuclear function, gain of cytoplasmic function, or both? Important cytoplasmic functions have been described for other nuclear RBPs that shuttle between nucleus and cytoplasm. Moreover, TDP-43 binds to a subset of mRNA 3'UTRs, a common binding region for RBPs that regulate translation, and TDP-43 protein was found to associate with polyribosomes under certain conditions (e.g. stress). Thus, TDP-43 might conceivably promote disease by directly affecting either general translation rates or the translation of specific mRNAs. To explore this possibility, we first examined the impact of altered TDP-43 expression on general translation in motor neuron-like cells. Using polysome profiling and nascent protein synthesis measurements, we found no effect on general translation after TDP-43 knockdown. Additionally, no effects on general translation were found upon expression of human TDP-43 (hTDP-43), hTDP-43 targeted to the cytoplasm or specific mutant variants of hTDP43. To investigate possible effects on translation of specific mRNAs, we used qRT-PCR to analyze the distribution of candidate TDP-43 targets across polysome profiles generated from either TARDBP or control siRNA treated

motor neuron-like cells. For the subset of mRNAs that we analyzed, including several mRNAs bound at the 3'UTR by TDP-43, no alteration was detected. Taken together, our results imply that neither loss nor gain of TDP-43 function has a significant impact on general translation in motor neuron-like cells under standard conditions. If TDP-43 does alter translation and thereby contribute to disease, the conditions under which this occurs and the specific mRNAs involved remain to be identified.

Disclosures: K. Miller: None. **K. Duncan:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.05/Q17

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Contract N01-AI-30063

NIH grant 1U54 AI-065357-04

Title: Motor function deficits identified in motor neurons of the spinal cords of mice infected with West Nile virus and other encephalitic viruses using optogenetic photoactivation

Authors: *J. D. MORREY, H. WANG, V. SIDDHARTHAN, J. O. HALL, K. K. KESLER; Inst. for Antiviral Reseach, Sch. of Vet. Med., Utah State Univ., LOGAN, UT

Abstract: Acute flaccid paralysis, limb weakness, respiratory insufficiency, and long-term motor function deficits are serious outcomes of human West Nile neurological disease (WNND) and of other viral encephalitides. Mouse models would be particularly useful in determining the physiological and cellular mechanisms of these motor function deficits; however, the development of such rodent models has been difficult due to the confounding effects of lethargy, neurocognitive or memory deficits, or otherwise a lack of motivation by animals to perform behavioral tests. These limitations have been overcome with the use of optogenetic transgenic mice expressing channelrhodopsin 2/EYFP fusion protein from the choline acetyltransferase promoter. Electromyography (EMG) of muscles of the diaphragms, forelimbs, and hind limbs have been activated by photoactivation of these transgenic mice in the C3-4 cervical cord, C4-5 cervical cord, and in the lumbosacral/cauda equina cord, respectively. Photoactivation-induced EMGs at these locations were markedly suppressed when these mice were infected with WNV. Suppression of diaphragmatic EMGs was associated with infection of ventral neurons, and loss of phrenic neurons in the cervical cord. Using a cannula mount on the lumbosacral cord,

hindlimb weakness can be monitored longitudinally. Transgenic mice infected in the cerebral spinal fluid with WNV, Japanese encephalitis virus, or neuroadapted Sindbis virus develop hindlimb motor function deficits as measured by optogenetic photoactivation. This optogenetic model will allow investigations into the mechanisms of viral induced motor function deficits, and for the evaluations of amyotrophic lateral sclerosis or spinal cord injury drugs for treatment of WNND and other viral encephalitides.

Disclosures: J.D. Morrey: None. **H. Wang:** None. **V. Siddharthan:** None. **J.O. Hall:** None. **K.K. Kesler:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.06/Q18

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIRG-11-204281

RAG12485

Title: The ALS disease associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons

Authors: *W. WANG, L. LI, W. LIN, X. WANG;
Case Western Reserve Univ., Cleveland, OH

Abstract: Mutations in TDP-43 lead to familial ALS. Expanding evidence suggest that impaired mitochondrial dynamics likely contribute to the selective degeneration of motor neurons in SOD1-associated fALS. In this study, we investigated whether TDP-43 mutations may impact mitochondrial dynamics and function. We demonstrated that overexpression of wild type TDP-43 reduced mitochondrial length and density in neurites of primary motor neurons which was further exacerbated by ALS associated TDP-43 mutants Q331K and M337V. In contrast, suppression of TDP-43 significantly increased mitochondrial length
and density in neurites, suggesting a specific role of TDP-43 in regulating mitochondrial dynamics. Surprisingly, both TDP-43 overexpression and suppression impaired mitochondrial movement. We further showed that abnormal localization of TDP-43 in cytoplasm induced devastating and widespread abnormal mitochondrial dynamics. TDP-43 co-localized with mitochondria in motor neurons and their colocalization was enhanced by ALS associated mutant. Importantly, co-expression of Mfn2 could abolish TDP-43 induced mitochondrial dynamics

abnormalities and mitochondrial dysfunction. Taken together, our data suggested that mutant TDP-43 impairs mitochondrial dynamics through enhanced localization on mitochondria, which causes mitochondrial dysfunction. Therefore, abnormal mitochondrial dynamics is likely a common feature for ALS and might be potential new therapeutic targets for ALS.

Disclosures: W. Wang: None. **L. Li:** None. **W. Lin:** None. **X. Wang:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.07/R1

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CIHR

Department of Laboratory Medicine and Pathobiology, University of Toronto

Title: Translational profiling in a TDP-43 transgenic mouse model of amyotrophic lateral sclerosis

Authors: *L. MACNAIR[1,2], B. ZHAO[1,2], D. MILETIC[1,2], M. GHANI[1,2], E. ROGAEVA[1,2], J. KEITH[1,3], L. ZINMAN[1,3], J.-P. JULIEN[4,5], J. ROBERTSON[1,2];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Ctr. for Res. in Neurodegenerative Dis., Toronto, ON, Canada; [3]Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; [4]Psychiatry and Neurosci., Laval Univ., Laval, QC, Canada; [5]Ctr. de Recherche du Ctr. Hospitalier Universitaire de Quebec, Laval, QC, Canada

Abstract: Amyotrophic lateral sclerosis (ALS), is an adult-onset neurodegenerative disease caused by the progressive loss of motor neurons in the CNS. A major feature of degenerating motor neurons in ALS is the mislocalization of the transactive response DNA-binding protein of 43kDa (TDP-43) from the nucleus to the cytoplasm, forming ubiquitinated inclusions. Mutations in TDP-43 account for a small portion of ALS cases, however, TDP-43 pathology is observed in over 90% of cases, indicating that abnormalities in TDP-43 are an important contributor to ALS pathogenesis.
Since TDP-43 is a nuclear DNA and RNA binding protein that has known functions in regulating RNA metabolism it is likely that abnormalities in TDP-43 will be reflected in changes in RNA processing and expression. Our objective is to identify these changes as a means to understanding how abnormal TDP-43 contributes to ALS pathogenesis.
Typical approaches to identifying changes in RNA expression (transcriptional profiles) rely on

analyzing total mRNA pools from a tissue region or cell type. We used a novel technique entitled Translating Ribosome Affinity Purification (TRAP) to obtain mRNA directly being translated from spinal cord motor neurons of TDP-43A315T mice. Mice expressing an EGFP-tagged ribosomal protein, L10a under control of the choline acetyltransferase promoter were crossed with TDP-43A315T mice to facilitate affinity purification of translating mRNAs from motor neuron polysomes. Translational profiles were obtained via microarray analysis in symptomatic mice and compared to wildtype (WT) littermates. The Biological Networks Gene Ontology Tool (BiNGO) plugin in Cytoscape was used to identify overrepresented GO terms. Genes with a ≥ 2 fold change between TDP-43A315T and WT were validated using immunofluorescence. Translational profiles showed that 28 genes were significantly misregulated. BiNGO analysis demonstrated that there was overrepresentation of genes involved in RNA metabolic process (GO-ID 0016070, p=3.27E-02), immune response (GO-ID 0006955, p=3.39E-02), and regulation of mitotic recombination (GO-ID 0000019, p=3.27E-02) in the TDP-43A315T mice, all which are highly implicated in ALS pathogenesis and other neurodegenerative diseases. Of the 28 misregulated genes, 20 are mapped and 7 had a fold change of ≥ 2. Immunofluorescence in TDP-43A315T motor neurons was indicative of microarray and will be validated in patients samples. This discovery-based approach has, for the first time revealed translational changes in motor neurons of a TDP-43 mouse model and will provide a greater understanding of the mechanistic basis of ALS.

Disclosures: L. Macnair: None. **B. Zhao:** None. **D. Miletic:** None. **M. Ghani:** None. **E. Rogaeva:** None. **J. Keith:** None. **L. Zinman:** None. **J. Julien:** None. **J. Robertson:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.08/R2

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

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FWO grant

IWT grant

Title: The involvement of Notch in the non-cell autonomous pathogenesis of amyotrophic lateral sclerosis

Authors: A. H. NONNEMAN[1,2], T. PHILIPS[3], C. EYKENS[1], *R. R. VANDENBERGHE[4], W. ROBBERECHT[1,2,5];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a late-onset progressive neurodegenerative disease, mainly but not exclusively affecting the motor neurons in the spinal cord, brain stem and cortex. This degeneration results into fasciculations, progressive muscle weakness and atrophy, and ultimately paralysis. The course of disease is typically between 1 and 5 years after onset of symptoms and the denervation of the respiratory muscles and the diaphragm is in most cases the fatal event.
Studies using the mutant SOD1 G93A mouse model have shown that the pathogenesis of ALS is non-cell autonomous, meaning that non-neuronal cells contribute to motor neuron degeneration. Typically for ALS is the presence of very reactive microglia and astrocytes, the so-called microgliosis and astrocytosis respectively, determining disease progression. Recently, oligodendrocytes are found to actively contribute to the pathogenesis of ALS. Oligodendrocytes degenerate and are replaced by newly differentiated oligodendrocytes. However, these new oligodendrocytes appear to be dysfunctional, as a decrease in Myelin Basic Protein (MBP) indicates failure of myelination, and a reduced expression of Monocarboxylate Transporter-1 (MCT1) shows impairment of proper neurotrophic support.
As the Notch signaling pathway is known to regulate cell-cell communication, the aim of this study was to investigate the involvement of Notch in the context of non-cell autonomous glial cell contribution to motor neuron degeneration.
We modulated the Notch signaling pathway in the SOD1 G93A mice by means of tamoxifen-induced Cre-Lox mediated recombination. Tamoxifen was administered via oral gavage at the age of 60 days for 4 consecutive days. We found that modulating the Notch signaling pathway affects the reactivity of both astrocytes and microglia. In addition, modulation of Notch rescued the expression levels of both MBP and MCT1, suggesting that the Notch pathway is involved in proper functioning of oligodendrocytes in the mutant SOD1 G93A mice.
In conclusion, our data suggest that the Notch signaling pathway may play an important role in the non-cell autonomous component of the pathogenesis of ALS.

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Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

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Program#/Poster#: 243.09/R3

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: ALS Society of Canada

McFeat Family Fund

Michael Halls Foundation

Title: Cytoprotective effect of Rho guanine nucleotide exchange factor (RGNEF)

Authors: K. CHEUNG, C. DROPPELMANN, K. VOLKENING, I. CAMERON, *S. H. PASTERNAK, M. J. STRONG;
Univ. of Western Ontario, London, ON, Canada

Abstract: Background: Rho guanine nucleotide exchange factor (RGNEF) is a dual-functionality protein having an RNA-binding domain and a GEF domain. Similar to other ALS-related RNA-binding proteins such as TDP-43 and FUS/TLS, RGNEF can form pathological neuronal cytoplasmic inclusions in ALS spinal motor neurons. It is unknown whether RGNEF inclusions lead to a loss of the normal function of RGNEF including a failure of RhoA activation and the accompanying response to cellular stress. In order to understand this, we are first characterizing the activity of RGNEF in response to cellular stress, including its ability to become incorporated in stress granules.
Methods: HEK293T cells were transfected (both as a transient and stable transfection) with myc-tagged RGNEF or empty vector (pcDNA myc-HisA). Cells were plated in 96-well plates, and then, 24 hours post plating, time-course experiments (ranging from 6 to 24 hours post-treatment) were conducted using heat shock (42.5^{o}C), sodium arsenite treatment (0.5mM) or sorbitol treatment (400mM). Cell viability was examined using the MTT assay and the formation of either stress (TIA-1 immunoreactive) or transport (staufen immunoreactive) granules visualized by confocal microscopy.
Results: For each of the three stressors examined, HEK293T cells over-expressing RGNEF showed increased survival when compared to pcDNA myc-HisA transfected cells. This cytoprotective effect was observed in both stable and transient transfections of myc-tagged RGNEF. At 2 and 4 hours post stress (sodium arsenite and sorbitol, respectively), stress granule formation was markedly up-regulated compared to non-stressed cells. In neither scenario was RGNEF observed to be associated with TIA-1 immunoreactive stress granules. In contrast, RGNEF did co-localize with transport granules.

Conclusion: These results suggest that in response to cellular stress, RGNEF expression is associated with cytoprotection and is not incorporated into stress granules, but rather into transport granules. These observations have important implications for our understanding of the consequences of sequestration of RGNEF within pathological neuronal cytoplasmic inclusions in ALS, where we hypothesize that it will be unavailable to participate in the physiologic stress response.

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Poster

243. **Motor Neuron Disease: Mechanisms I**

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Program#/Poster#: 243.10/R4

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant NS061867

NIH grant NS068179

Title: Reduced levels of a glycosylated species of the insulinoma-associated protein 2 (IA2) in ALS spinal cord: Evidence of dysregulated endosomal trafficking in ALS

Authors: *R. P. BOWSER, D. M. RIASCOS;
Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Several mutations linked to ALS implicate dysregulated endosomal trafficking as an important pathophysiological mechanism. Neurotransmission in motor neurons relies on normal functioning of transport of neuropeptides and active cell membrane components. We recently used unbiased proteomics methodologies to identify proteins that exhibit abnormal levels in the CSF of ALS patients. We detected a decrease of the Insulinoma-Associated protein 2 (IA2) in the CSF of ALS patients. IA2 is a transmembrane protein belonging to the protein tyrosine phosphatase superfamily and appears enriched in secretory vesicles and dense-core granules of various neuroendocrine cell types including central nervous system neurons. IA2 is processed to mature form via known posttranslational modifications. Thus, we characterized the expression, distribution, and biochemistry of IA2 in ALS by using three antibodies that bind distinct epitopes to recognize all IA2 species. Spinal cord, hippocampus and frontal cortex tissue sections from ALS, FTLD and age-matched controls were subjected to IA2 immunohistochemistry (IHC). Snap frozen tissue from ALS and control subjects were used for western blot analysis. We also

compared IA2 expression between human tissue and Neuro2A, SH-SY5Y, and HEK-293 cell lines. We observed reduced levels of IA2 immunoreactivity in the ALS spinal cord and different IHC patterns when using the 3 IA2 antibodies. Subsequently, we demonstrated that IA2 also underwent posttranslational modifications in spinal cord in agreement to prior reports in neuroendocrine tissues. However, only the high molecular weight (130 kDa) species of IA2 was reduced in ALS when compared to controls. The 130 kDa IA2 species results from glycosylation of specific resides in the pro-IA2 species. Thus, our data indicate altered processing and metabolism of IA2 during ALS. Our findings suggest reduced IA2 function in ALS, since glycosylation is a critical posttranslational modification involved in the protein-protein interactions and function of IA2. Therefore, decreased levels of this particular species may indicate dysfunction in intracellular sorting of cargoes and instability of specialized membrane domains (such as neuromuscular junction) which in turns contributes to motor neuron vulnerability in ALS.

Disclosures: R.P. Bowser: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iron Horse Diagnostics, Inc.. **D.M. Riascos:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.11/R5

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: Dysregulation of neurofilament composition underlies disease-causing SOD1-mediated motor neuron degeneration

Authors: *H. CHEN[1], K. QIAN[1], Z. DU[2], J. CAO[2], L. W. BLACKBOURN, IV[2], A. ERRIGO[2], H. CINDY[2], Y. YIN[2], J. LU[2], M. AYALA[2], S.-C. ZHANG[2];
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Abstract: Neurofilament (NF) tangle is a pathological hallmark of amyotrophic lateral sclerosis (ALS), including that caused by mutations in the Cu/Zn superoxide dismutase (SOD1) gene. Transgenic expression of multiple copies of the mutant forms of SOD1 in animals indeed leads to aggregation of proteins including neurofilaments (NF) and motor neuron degeneration although mechanisms underlying NF aggregation and selective motoneuron degeneration remain unknown. Here we show that NF aggregation appeared progressively in spinal motor neurons but rarely in non-motoneurons that were derived from induced pluripotent cells (iPSCs) from

patients with SOD1 (D90A and A4V) mutations at a time when glial cells have not developed. This pathological change was accompanied by reduced expression of neurofilament-low (NF-L) and altered proportion of NF subunits. Such motoneuron NF changes were mimicked by expression of a single copy of the same mutant SOD1 in human embryonic stem cells (ESCs) and prevented by genetic correction of the SOD1 mutation in the patient's iPSCs. Importantly, conditional expression of NF-L in the SOD1 iPSC-derived motor neurons corrected the NF composition and mitigated NF aggregation. These results indicate that the mutant SOD1 alters the NF composition and results in NF aggregation in motor neurons and that the degenerative process can occur in a cell-autonomous manner. The faithful modeling of ALS pathology highlights the utility of the stem cell strategy for revealing pathological processes of neurological conditions under the human genetic background.

Disclosures: H. Chen: None. **K. Qian:** None. **Z. Du:** None. **J. Cao:** None. **L.W. Blackbourn:** None. **A. Errigo:** None. **H. Cindy:** None. **Y. Yin:** None. **J. Lu:** None. **M. Ayala:** None. **S. Zhang:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.12/R6

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Paul and Harriett Campbell Fund for ALS Research

Zimmerman Family Love Fund

ALS Association, Greater Philadelphia Chapter

Title: Serum ferritin is elevated in amyotrophic lateral sclerosis patients

Authors: *J. R. CONNOR[1], X. W. SU[1], S. L. CLARDY[2], R. J. LAWSON[3], H. E. STEPHENS[2], Z. SIMMONS[2];
[1]Neurosurg., [2]Neurol., Penn State Col. of Med., Hershey, PA; [3]Northeast ALS Consortium, Boston, MA

Abstract: Background: Dysregulated iron metabolism contributes to ALS pathophysiology. HFE iron regulatory gene polymorphisms may increase ALS risk, and iron dyshomeostasis negatively impacts pathways implicated in ALS, including oxidative stress.
Objectives: To analyze levels of serum ferritin, a marker of iron metabolism, in ALS patients as

well as healthy and diseased controls, and to determine if ferritin impacts survival.
Methods: An IRB-approved, retrospective analysis was performed of data from 137 ALS patients, 152 healthy controls, and 80 patients with other neurological diseases seen at a university-based multidisciplinary ALS clinic or who provided samples to the Northeast ALS Consortium. Gender, age, site of onset, and, for select patients, dates of symptom onset and death were recorded. Survival time was defined as the duration from symptom onset to death. Serum ferritin levels were measured using standard clinical laboratory procedures. Ferritin levels between groups was compared using ANOVA. Spearman correlation was used to analyze the association between ferritin and survival time. Ferritin levels were categorized into high and low groups, and survival was analyzed using Kaplan-Meier logrank statistics and Cox proportional hazards regression.
Results: Age differed in ALS patients (mean 60.5 years) compared to healthy (mean 44.1) and diseased controls (mean 52.1). However, age was not associated with serum ferritin levels (R-squared 0.063). Gender proportions differed in ALS patients (65.3% males) compared to healthy (32.5% males) and diseased controls (38.5% males), and gender significantly affected ferritin levels (mean 224.8 ng/ml, males; mean 90.0 ng/ml, females; $p < 0.001$). Gender stratification was performed. In males, ferritin was significantly higher in ALS patients (mean 286.6 ng/ml) than either healthy (mean 160.8 ng/ml, $p < 0.001$) or diseased controls (mean 164.5, $p = 0.003$). In females, ferritin was significantly higher in ALS patients (mean 142.6 ng/ml) than either healthy (mean 69.3 ng/ml, $p < 0.001$) or diseased controls (mean 77.5, $p < 0.001$). Ferritin was not associated with survival time in either males (R-squared 0.001) or females (R-squared 0.069). ALS patients were categorized into low and high ferritin groups by median values (200.0 ng/ml, males; 123.0 ng/ml, females). Ferritin status did not significantly impact survival by Kaplan-Meier logrank tests or Cox regression in either gender.
Conclusions: These results suggest altered iron metabolism in ALS patients, an effect not simply due to neurological disease. However, serum ferritin alone may be insufficient to reflect or predict disease progression and survival.

Disclosures: J.R. Connor: None. **X.W. Su:** None. **S.L. Clardy:** None. **R.J. Lawson:** None. **H.E. Stephens:** None. **Z. Simmons:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.13/R7

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Cynthia and George Mitchell Foundation

Cullen Trust for Health Care

Title: Phenotypic analysis of neurodegeneration induced by codon optimized wild type TDP-43 in a *Drosophila* model of ALS

Authors: *T. YUSUFF, S. CHATTERJEE, G. R. JACKSON;
Univ. of Texas Med. Br., Galveston, TX

Abstract: TAR DNA Binding Protein-43 (TDP-43) is known to mediate neurodegeneration associated with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). The exact mechanism by which TDP-43 exerts toxicity in patient brains remains unclear. In a Drosophila model, we have identified robust gain of function phenotypes produced by misexpression of insect codon-optimized wild type TDP-43 in retina using the binary GAL4/UAS system, as well as direct promoter fusion constructs. Compared to human transgenic flies, the codon optimized flies express more protein. We were able to detect higher molecular weight species in SDS-PAGE under non-denaturing conditions in the codon-optimized lines. Both nuclear and cytoplasmic expression of TDP-43 were detected along with cytoplasmic aggregation. Our observation led us to believe TDP-43 has the propensity to form toxic protein aggregates when too much protein is present via a gain of function mechanism. Using the direct promoter fusion constructs, we identified that in adult retina TDP-43 misexpression causes strong depigmentation, disruption of polarity in interommatidial bristles, and apparent necrotic patches or hypermelanization that worsen with age. Specifically, the photoreceptor neurons appear flattened and shorter in length with failure of rhabdomere separation; they contain large vacuole-like structures that are morphologically similar to autophagic intermediates. Furthermore, misexpression of TDP-43 in the CCAP expressing neurons in ventral nerve cord leads to wing expansion defects and swelling. When expressed in the Drosophila notum using Eq-GAL4, TDP-43 causes loss of bristles. Based on our observation, we believe that TDP-43 is exerting its toxicity via gain of function mechanism. Further investigation will help us identify and better understand the exact disease mechanism of TDP-43 proteinopathies and find potential therapeutic targets.

Disclosures: T. Yusuff: None. **S. Chatterjee:** None. **G.R. Jackson:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.14/R8

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Dysautonomia Foundation

NIH R01 NS35714

Title: Cognitive phenotype in a mouse model of Familial Dysautonomia: Spatial memory

Authors: S. POWELL[1], F. LEFCORT[2], *J. L. WIESELER[3], A. BABCOCK[1];
[1]Psychology, [2]Cell Biol. and Neurosci., [3]Dept. of Psychology, Montana State Univ., Bozeman, MT

Abstract: Familial dysautonomia (FD) is a Hereditary Sensory and Autonomic Neuropathy (Type III) marked by a mutation within the IKBKAP gene encoding the IKAP protein. IKAP is an essential component in growth and development of the sensory and autonomic nervous systems. This protein is widely expressed throughout the nervous system including the hippocampus. Symptoms of FD are progressive and include delayed motor development, psychological disorders, and mild cognitive impairment (Axelrod, 2006). The Lefcort lab group has generated a mouse model of FD in which *Ikbkap* was selectively deleted from CNS neurons. The present study assessed spatial memory of FD mice using an object location task. FD mice and age-matched controls were tested in a task that included habituation, acquisition, and testing phases. Mice were habituated to the apparatus in the absence of the test objects for 10 mins. During acquisition, mice were exposed to two identical objects for 10 mins. One hr after acquisition, animals were reintroduced to the apparatus with one of the object placed in a different location (testing phase). Exploration of the moved object during the testing phase represented a measure of recognition and memory of the spatial change. Time spent within one cm of the moved object in proportion to total object exploration was used for analysis. Other behaviors were recorded and analyzed separately (ie., locomotor activity, mobility and total object exploration).
There was no significant difference in the proportion of exploratory behavior for the novel location object between FD and control groups. However, FD subjects explored objects significantly more than controls. Additionally, FD mice spent significantly less time immobile (e.g. FD mice were more active). Taken together, these data suggest that FD mice are not impaired in object spatial memory, but exhibit behaviors indicative of decreased anxiety response to novelty. Further research is under way to characterize the behavioral phenotype of this model of FD.

Disclosures: S. Powell: None. **F. Lefcort:** None. **J.L. Wieseler:** None. **A. Babcock:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.15/R9

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: AriSLA

SMA Europe

Title: Pathogenic FUS mutations retain spliceosomal snRNPs in the cytoplasm

Authors: V. GERBINO[1,2], A. MIRRA[3], *N. CANU[4], M. CARRÌ[1,2], M. COZZOLINO[5], T. ACHSEL[6];
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Abstract: Motor neuron diseases such as Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA) are associated with defects in proteins involved in RNA metabolism (TDP43 and FUS, and SMN, respectively). SMN, the causative factor in SMA, is crucial for the biogenesis of the spliceosomal snRNPs. FUS is a nuclear protein which has been implicated in alternative splicing or mRNA localisation; it forms cytoplasmic aggregates, as a consequence of disturbed nuclear import due to disease-causing mutations. It is extremely likely that the cytoplasmic aggregates are cytotoxic because they trap important factors; the nature of these factors, however, remains to be elucidated. Based on our previous findings, showing that FUS and SMN associated with each other, and that FUS binds to spliceosomal snRNPs, we investigated whether aggregation-prone FUS mutants might interfere with the localisation and biogenesis of snRNPs. Indeed, in transfected mouse motoneuronal-like NSC34 cells, mutations in FUS do not affect its association to the snRNPs. However, mutant FUS and SMN co-localise in multiple cytoplasmic aggregates, and snRNAs are retained into the cytoplasm. As a result, alterations in the alternative splicing of a reporter plasmid are observed. Alternative splicing defects as well as alterations in SMN sub-cellular distribution are also observed in cells depleted of FUS by RNA interference, suggesting that aggregated mutant FUS might disturb the nuclear import of SMN, thus reducing the availability of functional snRNPs in the nucleus. Experiments are undergoing to further characterise this issue. Overall, our observations indicate that FUS mutations and genetic depletion of SMN converge onto the same pathway, i.e. alternative splicing changes, that might represent a unifying theme in the FUS-related ALS and SMA.

Disclosures: V. Gerbino: None. **N. Canu:** None. **M. Carrì:** None. **A. Mirra:** None. **M. Cozzolino:** None. **T. Achsel:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.16/R10

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: 1RO1NS056314

Title: Lis1 and Ndel1 regulate dynein-dependent mitochondria traffic in mature neurons

Authors: *L. SHI[1], J. PANDEY[2], D. SMITH[1];
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Abstract: Lissencephaly (smooth brain), caused by Lis1 haploinsufficiency, is a rare brain malformation characterized by defective neuronal mitosis and migration during the early weeks of gestation. The disorder results in increasingly severe seizures and ultimately childhood lethality. If the well-characterized developmental defects are solely responsible for the seizures and lethality, then treatment options are limited. However, Lis1 expression remains high in the adult nervous system. Lis1 binds to cytoplasmic dynein, a microtubule motor critical for retrograde axonal transport. In earlier studies, we demonstrated a role for Lis1 and its interacting partner, Nudel, in dynein-dependent lysosome transport in adult rat sensory neurons. Because mitochondria play important role in cell survival and energetics, we have now addressed the question of whether Lis1 and Nudel also regulate dynein-dependent mitochondria transport in mature axons. As with lysosomes, Lis1 overexpression increased in the percentage of mitochondria moving in a retrograde direction, while Lis1 knockdown reduced retrograde motility. Similarly, loss of both Lis1 and Nudel dramatically reduced motility of both organelle types. However, there were major differences in the impact of Lis1 point mutants on mitochondria transport compared to lysosome transport. A Lis1 mutant that blocks dynein binding had severe effects on motility in both organelle systems, while a Nudel-binding mutant had a much more severe phenotype with respect to mitochondria. Our current model is that while Nudel and Lis1 must both be present for efficient lysosome and mitochondrial transport, a direct interaction between Lis1 and Nudel is more important for the larger mitochondria. From a disease standpoint, disruption of the Lis1/Nudel dynein regulatory pathway could result in post-developmental defects associated with faulty mitochondrial distribution.

Disclosures: L. Shi: A. Employment/Salary (full or part-time):; University of South Carolina. 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.; 1RO1NS056314. **J. Pandey:** A.

Employment/Salary (full or part-time):; Harvard Medical School. **D. Smith:** A. Employment/Salary (full or part-time):; University of South Carolina.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.17/S1

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS051419

NIH Grant NS062055

Robert Packard Center for ALS Research

Title: Study of mitochondria quality control in the G93A-SOD1 mouse model for ALS

Authors: G. M. PALOMO, J. MAGRANE, B. SIDER, *G. MANFREDI;
Weill Med. Col. Cornell Univ., NEW YORK, NY

Abstract: Amyothropic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease caused by specific degeneration of motor neurons. Familial forms of ALS represent a 10% of the all the cases of this disease and mutations in the gene Cu-Zn superoxide dismutase (SOD1) account for a 20% of these. Several mechanisms have been proposed to explain the pleiotropic pathogenic effects of mutant SOD1, among which mitochondrial dysfunction is considered a central component of the pathogenic mechanisms underlying motor neurons degeneration. Mitochondria quality control in neurons is a tightly regulated and critical pathway, whose ultimate purpose is the specific degradation of damaged mitochondria. Elimination of damaged mitochondria occurs largely through the mitophagy pathway, and deregulation of this process can lead to accumulation of dysfunctional mitochondria. We studied mitochondria quality control in the G93A-SOD1 mouse model of ALS using either enriched-mitochondria fractions from spinal cord or primary cortical neurons obtained from E18 embryos. On mitochondria fractions, at disease end stage, we found increased levels of several markers of autophagy in mutant samples compared to non-transgenic littermates: LC3-II protein levels were markedly higher and p62 was accumulated on mitochondria. At disease onset, the increase in LC3-II associated with mitochondria was less marked, suggesting a progression of this molecular phenotype. In SOD1 mutant primary cortical neurons in basal conditions we also found an increase in LC3-II protein levels. To evaluate the autophagy flux in this system, neurons were exposed to bafilomycin A1 (vacuolar ATPase inhibitor). The results indicated a robust increase

in autophagy flux in mutant cells, measured as the rate of accumulation of p62. Also, the different steps of the autophagy process are currently being examined in cortical neurons by using fluorescent-labeled proteins and live-cell imaging techniques. The presence of dysfunctional mitochondria in neuromuscular junctions and motor neurons of ALS models has been extensively described and could be caused by accumulation of damaged organelles that fail to be turnover correctly. Further studies to assess the efficiency of autophagy at these target sites of neurodegeneration are needed in order to determine if the accumulation of damaged mitochondria is the consequence of defective autophagy machinery or of excessive mitochondrial damage that saturates the system.

Disclosures: G.M. Palomo: None. **J. Magrane:** None. **B. Sider:** None. **G. Manfredi:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.18/S2

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: The Farber Family Foundation

Title: Studying the function of Fused in sarcoma (FUS) at the presynaptic terminal of the neuromuscular junction: Implications for FUS-mediated ALS

Authors: *K. KRISHNAMURTHY, M. SHAHIDULLAH, I. LEVITAN, D. TROTTI, P. PASINELLI;
NEUROSCIENCE, THOMAS JEFFERSON UNIVERSITY HOSPITAL FOR NEUROSCIENCE, PHILADELPHIA, PA

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that leads to fatal paralysis. About 10% of ALS cases are familial (fALS), and are associated with mutations in one or another of several genes, while the remainder are sporadic (sALS). FALS and sALS are clinically indistinguishable, suggesting that the different forms of the disease converge on common pathways. Destruction of the neuromuscular junction (NMJ) is a hallmark (and converging point) of all forms of ALS. One gene associated with fALS encodes the DNA/RNA binding protein Fused in Sarcoma (FUS), and FUS-related *Drosophila* models of ALS show early functional NMJ abnormalities as in human disease. In particular loss of the FUS homologue Cabeza disrupts the NMJ suggesting a role for FUS in formation of synapses at the neuromuscular junction (NMJ). In this study, we investigated the role of FUS at mammalian NMJ using a co-culture of rat motor neurons with myotubes. We first examined the localization

of endogenous FUS with pre-and-postsynaptic markers. We find FUS primarily express at the presynaptic terminal of the NMJ with a striking co-localization to a presynaptic marker, synaptic vesicle 2 (SV2), in motor neurons. FUS levels at postsynaptic densities associated with PSD-95 are significantly lower. Studies are underway to characterize the functional consequences of presynaptic FUS at the NMJ as well as the mechanisms by which disease-causative mutant FUS, through perhaps a dominant-negative mechanism, impairs the normal function of FUS and neurotransmitter release at NMJ, thereby triggering atrophy on the contacted muscle cells.
Funding: The Farber Family Foundation

Disclosures: K. Krishnamurthy: None. **M. Shahidullah:** None. **I. Levitan:** None. **D. Trotti:** None. **P. Pasinelli:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.19/S3

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS036081

NIH Grant NS69212

Title: Muscle fiber-type regulation of maintenance of neuromuscular junction innervation

Authors: *C. MILLIGAN[1], J. STRUPE[2], C. MANSFIELD[2], M. ROBINSON[2], R. OPPENHEIM[2], A. ZAIA RODRIGUES[2], R. KUDO[2];
[1]Wake Forest Sch. Med., WINSTON SALEM, NC; [2]Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Motoneurons (MNs) are the largest and maybe the most complicated neurons. They must coordinate interactions of the soma and dendrites with the CNS environment and those of the axon and terminals exposed to the peripheral environment. Much amyotrophic lateral sclerosis (ALS) research has focused on pathology in the spinal cord; however, effective treatment strategies for ALS will involve targets in the spinal cord and at the neuromuscular junction (NMJ). In cultured MNs the JNK signaling pathway appears to contextually regulate both survival and degeneration. To further investigate how JNK may regulate MN development and survival in vivo, we utilized JNK isoform knock-out (KO) mice. We found no difference in MN cell death or innervation between KO and non-transgenic, wild-type (WT) mice. In JNK1 KO mice, however, we found deficits in motor behavior accompanied by muscle denervation

beginning at postnatal day (P) 180. We examined denervation in the tibialis anterior (TA) muscle that is composed of fast fatigue resistant fibers [type 2a (F)] and fast fatigable [type 2b (FF)] fibers and the soleus muscle that is composed of slow [type 1 (S)] fibers. The NMJs of the TA had significant denervation at P180 while denervation in the soleus muscle was not significant until 1.5 years. Interestingly, in the SOD1 mouse, MNs that innervate FF fibers denervate NMJs first, whereas those that innervate S fibers do not appear to undergo denervation until very late stage of the disease. Both results are intriguing when we consider the following: 1) both TA and soleus motor pools in JNK1 KO mice are deficient in JNK1 signaling and appear to develop and innervate their target muscles normally; 2) in the SOD1G93A mouse model of ALS both TA and soleus motor pools exhibit mitochondrial pathology, altered afferent input and a decreased capillary density in the spinal cord; 3) yet, in both mouse models, MNs innervating the TA denervate their NMJs significantly before denervation by the soleus MNs. This striking difference in target denervation may suggest that differing muscle physiologies influence the rate, extent, and stability of innervation with age. We have preliminary data that suggest differences in expression of MN survival promoting factors (BDNF and heat shock protein (Hsp) 70) have decreased expression in fast muscles as compared to slow muscle fibers. If and how expression of MN survival promoting factors is altered in JNK1 deficient animals is under investigation. Understanding the molecular differences of these peripheral (muscle derived) signals in the pathology of MN disease may provide a foundation for therapeutic development.

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Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.20/S4

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Title: G93A mutated SOD1-linked murine ALS produces abnormal exocytotic release of glutamate

Authors: *C. USAI[1], M. MILANESE[2], T. BONIFACINO[2], F. ONOFRI[2], L. MUSAZZI[3], F. BENFENATI[2,4], M. POPOLI[3,5], G. BONANNO[2,6];
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Abstract: Glutamate (Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons in amyotrophic lateral sclerosis (ALS) and reduced astrocytary glutamate transport, which in turn increases the synaptic availability of the amino acid neurotransmitter, was suggested as a cause. Alternatively, here we report our studies on the exocytotic release of glutamate as a possible source of excessive glutamate transmission.
We have studied the release of [3H]D-aspartate and of endogenous Glu induced by depolarizing and non depolarizing stimuli, known to induce exocytotic neurotransmitter release, in mice expressing human SOD1 with the G93A substitution [SOD1G93A], a transgenic model of ALS, respect to mice expressing the unmodified human SOD1 and to non transgenic littermates. Exposure to 15 or 25 mM KCl or to 0.3 or 1 μM ionomycin provoked an almost complete Ca2+-dependent release of Glu from spinal cord synaptosomes. The exocytotic release of Glu induced by KCl or ionomycin was dramatically increased in symptomatic SOD1G93A mice than in controls. The higher glutamate release in mutant animals was already present in early-symptomatic 70-90 and pre- symptomatic 30-40 day-old mice. Noticeably, the stimulus-evoked release of [3H]GABA or [3H]Glycine in spinal cord of SOD1G93A mice did not differ from controls
As to the molecular determinants of this abnormal glutamate exocytosis, increased Ca2+ levels were detected in SOD1G93A mouse spinal cord nerve terminals, accompanied by increased activation of Ca2+/calmodulin-dependent kinase II and increased phosphorylation of synapsin I. In line with these findings, release experiments suggested that the glutamate release augmentation in SOD1G93A mice involves the readily releasable pool of vesicles and a greater capability of these vesicles to fusion upon stimulation. Modifications of the SNARE complex and of release proteins were also observed.
Our in vitro results show that the exocytotic release of Glu is enhanced in mutant SOD1 mice. If it occurs in vivo, the different modulation of Glu, GABA and glycine release here reported could induce an unbalance between spinal inhibitory and excitatory transmission in ALS.

Disclosures: C. Usai: None. **M. Milanese:** None. **T. Bonifacino:** None. **F. Onofri:** None. **F. Benfenati:** None. **G. Bonanno:** None. **L. Musazzi:** None. **M. Popoli:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.21/S5

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NSO61867

NIH Grant NSO80614

Title: The RNA binding protein RBM45 associates with nuclear stress bodies during cellular stress events

Authors: *M. A. COLLINS[1], R. RICE[2], R. BOWSER[2];
[1]Neurosci., Univ. of Pittsburgh, Phoenix, AZ; [2]Neurol., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: The RNA binding protein RBM45 is a component of the inclusion bodies found in neurons and glia in neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), and Alzheimer's disease (AD). In human neurodegenerative disease post-mortem tissue, RBM45 also exhibits a speckled nuclear staining pattern that is independent of inclusion bodies. To investigate the mechanisms of inclusion formation and the biological function of RBM45-positive nuclear granules, we performed immunocytochemistry and co-immunoprecipitation experiments in cultured cells. A variety of subnuclear structures exhibit speckled nuclear staining patterns and we used a combined iterative digital deconvolution/quantitative immunocolocalization analysis to characterize the presence or absence of RBM45 in these structures. We found that RBM45 does not colocalize with markers of nuclear speckles (SC35), Cajal bodies (coilin), or nuclear gems (SMN) and likewise is not a component of cytoplasmic stress granules. In untreated cells, RBM45 was diffusely localized throughout the nucleus, with few granules present. In contrast, when cells were subjected to heat shock or genotoxic stress, the number and size of RBM45-positive granules significantly increased ($p < 0.05$). We identified significant colocalization of RBM45 and the nuclear stress body-associated proteins HSF1 and SAM68 in stressed cells ($p < 0.05$). Co-immunoprecipitation experiments demonstrated that RBM45 could be pulled down using anti-HSF1 antibodies and HSF1 could be pulled down using anti-RBM45 antibodies. Collectively, these results demonstrate that RBM45 is a new component of nuclear stress bodies and identify a new potential therapeutic target for neurodegenerative disorders such as ALS, FTLD, and AD.

Disclosures: M.A. Collins: None. **R. Rice:** None. **R. Bowser:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.22/S6

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: FAPESP

CNPq

Title: Broker genes in the skeletal muscle of SOD1G93A amyotrophic lateral sclerosis mouse model

Authors: *J. R. MAXIMINO[1], G. P. OLIVEIRA[1], M. MASCHIETTO[2], D. M. CARRARO[2], E. ZANOTELI[1], G. CHADI[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of motor neurons (MN). The events regarding MN death are still unknown. Evidences point to skeletal muscle as a primary target of ALS, however little is known about the signaling mechanisms of the disease. In a previous study, the web tool http://bioinfo.lbhc.hcancer.org.br/interactomegraph/ was used to obtain the interactome, and mathematical analysis was applied in order to find broker genes from a gene expression microarray analysis of Wnt and related pathways in gastrocnemius from SOD1G93A mouse model and its age-paired wild-type controls in the age of 40 and 80 days. The network based on the genes was composed by 251 genes from the 40 days and 531 genes from 80 days, where the Grb2 and Src were key genes for the construction of both networks.
In order to better understand the involvement of Grb2 and Src in the presymptomatic phases of ALS, RNA and total protein of gastrocnemius of SOD1G93A mice at presymptomatic ages of 40 and 80 days and wild-type were extracted and the qPCR and western blotting were conducted. The results were analyzed by Student t-test.
Up regulations of the gene expression of Src (1.6 fold, $p<0.05$) and Grb2 (1.3 fold, $p>0.05$) were seen in 80 days old ALS mice compared to wild-type controls. The western blotting showed significant increases of the Src (9.6%) and Grb2 (42.6%) protein levels in the 80 days old ALS mice. Above described, changes in the gene expression and protein levels were not found in the 40 days old ALS mice.
These results showed that already in presymptomatic periods, there are important changes in gene expression and protein levels of skeletal muscle from ALS mice. The Src is important to stabilize the neuromuscular synapse and is considered as a hub for glutamate receptors regulation leading a modulation of synapse. Grb2 is involved in cell proliferation and transformation and via Erk is thought to influence the volume and number of cells, neuritis, and synapses.
Further studies are needed to better characterize whether signaling pathways of Src and Grb2 are involved in the physiopathology of ALS or in the responses to maintain muscular function in the presymptomatic phase of ALS.

Disclosures: J.R. Maximino: None. **G.P. Oliveira:** None. **M. Maschietto:** None. **D.M. Carraro:** None. **E. Zanoteli:** None. **G. Chadi:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

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Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: FAPESP Grant 2009/14214-7

FAPESP Grant 2010/20457-7

Title: Early gene expression changes in astrocytes from SOD1 Amyotrophic Lateral Sclerosis mouse model

Authors: *G. P. OLIVEIRA[1], J. R. MAXIMINO[1], A. F. CARVALHO[2], D. M. CARRARO[2], G. CHADI[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is an adult-onset and fast progression neurodegenerative disease that leads the loss of motor neurons. Familial ALS is manly linked to dominant mutations in the gene for Cu/Zn superoxide dismutase (SOD1). Toxic signs from non neuronal cells, mainly astrocytes and microglia, have been proposed to be involved in the rapid evolution of the disease. However, little is known about the related signaling mechanisms.
In order to better understand the triggering events of ALS, RNA of lumbar spinal cords of transgenic SOD1G93A ALS mice at pre-symptomatic ages of 40 and 80 days and age-paired wild type controls was extracted and the microarray analysis of the whole mouse genome was performed. Differentially expressed genes were identified by means of the Bioconductor packages “Agi4x44Preprocess” and “limma”. Gene Ontology analyses of differentially expressed mRNAs were conducted using the NCBI's online DAVID bioinformatics interface. qPCR was conducted in order to validate microarray data. Ventral horn astrocytes from 40 and 80 old days mice, labeled by immunofluorescence, were submitted to laser microdissection, the RNA was extracted and amplified for further evaluation of a selected gene by qPCR.
Statistical analysis has pointed to 492 differentially expressed genes (155 up and 337 down regulated) in 40 days old ALS mice and 1105 (433 up and 672 down regulated) in 80 days old ALS mice. At the age of 40 days, the main biological process regarding down regulated genes was synapse organization, while the up regulated process were fructose metabolism and regulation of neuronal synaptic plasticity. In 80 days old ALS mice, the main down regulated processes were mRNA metabolism, ribonucleoprotein complex biogenesis and blood circulation. Also, the up regulated genes were related to actin filament based process, vesicle mediated transport, protein modification by small protein conjugation, acetyl-coA metabolism and aerobic

respiration. qPCR have confirmed the microarray results. The appearance of protein modification process in 80 days old ALS mice motivated the evaluation of Ube2i gene expression in astrocytes of both ages. Ube2i was found up regulated both in 40 (5.52 fold, $p<0.05$) and 80 days (1.77 fold, $p <0.05$).
Preliminary results indicate important processes occurring in astrocytes of ALS mice since early 40 days. The Ube2i gene encodes the Ubc9 protein, an enzyme of sumoylation pathway. SUMO1-conjugated proteins are reported to promote astrogliosis in symptomatic ALS due to their accumulation in astrocytes nuclei. Further studies are being conducted in order to identify related processes that could clarify the ALS mechanisms.

Disclosures: G.P. Oliveira: None. **J.R. Maximino:** None. **A.F. Carvalho:** None. **D.M. Carraro:** None. **G. Chadi:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.24/S8

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: CIHR & ALS Canada JNM-123677

Title: Propagation of misfolded SOD1 in mouse models of ALS; Implications for mechanism of disease pathogenesis

Authors: *M. A. O'NEILL, L. I. GRAD, A. PETIT, A. J. ROSKAMS, N. R. CASHMAN; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: ALS is a fatal neurodegenerative disease caused by the loss of motor neurons, leading to progressive paralysis and muscle atrophy. Familial cases of ALS (FALS) account for ~10% of all cases, defining the remaining 90% as sporadic (SALS). Mutations in superoxide dismutase 1 (SOD1), occurring in ~2% of all cases of ALS, cause the protein to misfold resulting in a cytotoxic gain-of-function. Although the exact mechanism is unknown, cytotoxic misfolded SOD1 is involved in disease pathogenesis, with a remarkable specificity for motor neurons, even though SOD1 is ubiquitously expressed in many cell types. Excluding *de novo* events, ALS cases don't have mutations in the SOD1 gene, yet the clinical and europathological similarities to FALS suggest a common mechanism of disease pathogenesis. Previous work has shown that under oxidizing conditions, wild type SOD1 misfolds, and acquires the same cytotoxic properties as mutant SOD1. Distinct cell types have different sensitivities to oxidizing events, which could have significant implications in disease onset and pathogenesis. Since pathogenic SOD1 is

misfolded, our laboratory developed antibodies that specifically recognize epitopes only exposed if the protein is misfolded. Using these antibodies, we analyzed spinal cord from control, FALS and SALS patients. Our data clearly shows the presence of misfolded/oxidized SOD1 in the spinal cords of both FALS and SALS patients, and remarkably, the amount detected in SALS is tantamount to that in SOD1 FALS. This data strongly implicates misfolded SOD1 as a marker in all cases of ALS. To analyze this further, we are currently analyzing for the cell-type distribution (motor neurons, astrocytes, microglia, and oligodendrocytes) of misfolded SOD1 in mouse models of ALS and human ALS spinal cord to localize site of misfolding. *In vitro* experiments with conditioned media and serial passaging have shown that the misfolding of SOD1 can be propagated from one source of cells, through the media, to recipient cells. *Ex vivo* studies with spinal cord organotypic slice cultures aim to address the notion of cell-to-cell propagation of misfolded SOD1. And by applying the insights from the cell-type specific analysis to the *ex vivo* experimental paradigm, cell-type specific primary cultures may be applied to *ex vivo* tissue to analyze the relative importance of each cell type to disease pathogenesis and disease progression.

Disclosures: M.A. O'Neill: None. **L.I. Grad:** None. **A. Petit:** None. **A.J. Roskams:** None. **N.R. Cashman:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 243.25/S9

Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

CREST/JST

Title: FUS regulates alternative splicing patterns of Mapt by cooperating with PSF/SFPQ: a novel link between FUS and Tau in the pathogenesis of ALS and FTLD

Authors: *S. ISHIGAKI, Y. FUJIOKA, T. UDAGAWA, D. HONDA, M. KATSUNO, G. SOBUE;
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Abstract: FUS is linked to the pathogenesis of amyotrophic sclerosis (ALS), which is one of the most cruel neurodegenerative diseases. Mutations in FUS gene cause familial ALS/FTLD and the protein mislocalization to the cytoplasm in the affected neurons, which is also observed in

sporadic cases of ALS/FTLD. We found that FUS made a high-molecular weight complex in the nucleus of neurons and that was uncompleted in the ALS-associated FUS mutants. We identified splicing factor, proline- and glutamine-rich (PSF/SFPQ) as a component of the high-molecular complex of FUS and found that the interaction between FUS and PSF/SFPQ was affected by disease-associated mutation in FUS. These findings suggested that loss of FUS function on alternative splicing could lead to neuronal degeneration in FUS-associated ALS/FTLD. We next established the FUS-regulated transcriptome profiles of primary neurons using exon-sensitive microarrays and identified that FUS regulated the alternative splicing of Mapt exon10. This alternative splicing event results in three-repeats (RD3) and four-repeats (RD4) Tau isoform production and the ratio of RD4/RD3 was increased when FUS was silenced in neurons. Morphological abnormality of neurite was observed in FUS-silenced primary neurons and it was rescued by silencing of RD4. FUS-knock-down mice were generated by injecting adeno-associated virus (AAV) encoding shRNA against FUS into bilateral hippocampus. The mice showed obvious reduction of FUS and increased ratio of RD4/RD3 in bilateral hippocampus. Abnormal anxiety behavior was observed in FUS-knock-down mice compared to the control. Pathological specimen analysis showed the change of the ratio of RD4/RD3 in FTLD and other tauopathies such as progressive supranuclear palsy (PSP). Thus, our findings suggest a pathophysiological link between FUS and Tau in ALS/FTLD.

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Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

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Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH NINDS F32 NS063535

NIH NINDS NS054850

NIH NINDS NS077863

Title: Comparison of action potential evoked dendritic calcium entry in SOD1G93A and non-transgenic spinal motor neurons

Authors: *K. A. QUINLAN, J. B. LAMANO, J. SAMUELS, D. L. WOKOSIN, C. J. HECKMAN;
Physiol., Northwestern Univ. Feinberg Sch. of Med., CHICAGO, IL

Abstract: In amyotrophic lateral sclerosis (ALS), the largest (fast fatiguable) motor neurons which lack Ca2+ buffering proteins are the most vulnerable to degeneration. Previous studies in mutant SOD1 mouse models have shown that spinal motor neurons have significantly more dendritic branching than age-matched counterparts. Whether these added dendrites have similar levels of activity has not yet been examined. Therefore, in this study we characterized action-potential-evoked dendritic Ca2+ entry in juvenile (P4-11) motor neurons in transverse slices of lumbar spinal cord. Whole cell patch was performed with both Ca2+ Green-1 and Texas Red dextrans diluted in the standard internal solution of the electrode. Once a stable patch configuration had been established for 20 minutes, 2 photon Ca2+ imaging was implemented using line scans across the dendritic arborization. The Texas Red was included to control for variations in dye transport throughout the processes, and the peak signal of the averaged Ca2+ transient was collected for locations throughout the dendritic field. Over all the neurons recorded, dendritic diameter and path distance from the soma were significant factors related to the observed Ca2+ transients. More robust Ca2+ transients were observed in the proximal dendrites, with decreasing Ca2+ transients observed distally along the process. As dendritic distance from the soma is inversely correlated with dendritic diameter, Ca2+ transients were found to be largest in the proximal processes with greatest dendritic diameter and decreased in the distal processes where dendritic diameter is reduced. Within individual neurons, there was significant variation between processes in 8 of 9 neurons, suggesting that in Ca2+ entry is not uniform throughout motor neuronal compartments but rather is independently regulated. Finally, dendrites of SOD1G93A motor neurons had significantly smaller Ca2+ transients than non-transgenic motor neurons (N = 4 & 5, respectively). In conclusion, action-potential-evoked dendritic Ca2+ transients were highly variable from dendrite to dendrite, potentially due to localized regulation of ion channel expression or neuromodulation. Within the expanded dendritic field of SOD1G93A motor neurons, there was an overall reduction in Ca2+ transients, perhaps a mechanism to equalize overall calcium entry from the expanded dendritic arborization.

Disclosures: K.A. Quinlan: None. **J.B. Lamano:** None. **J. Samuels:** None. **D.L. Wokosin:** None. **C.J. Heckman:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

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Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R00NS061803

NIH Grant R01NS070899

AHA Postdoctoral Fellowship 12POST12030381

Title: Non-proteolytic ubiquitination of glycogen phosphorylase by the malin-laforin complex regulates glycogen metabolism

Authors: *M. S. GENTRY[1], V. DUKHANDE[2], B. PAASCH[2];
[1]Biochem., Univ. of Kentucky, LEXINGTON, KY; [2]Univ. of Kentucky, Lexington, KY

Abstract: Autosomal recessive mutations in genes encoding malin or laforin cause Lafora disease (LD). LD is a progressive myoclonic epilepsy characterized by pathological aberrant glycogen accumulations in brain and various other organs. Our group identified laforin as a novel glycogen phosphatase essential for maintaining glycogen in a soluble form. Additionally, we discovered that malin is a RING-type E3 ubiquitin ligase and demonstrated that it ubiquitinates proteins involved in glycogen metabolism such as laforin, glycogen synthase, protein targeting to glycogen and glycogen debranching enzyme. However, malin's role in LD pathogenesis is not completely understood. In this study, we aimed to discover substrate/s of malin and its function in LD pathogenesis. We utilized malin- and laforin-knockout mice, immunoprecipitations, Western analyses, immunocytochemistry, glucan-binding assay, PAS staining, and enzyme activity measurements. We discovered glycogen phosphorylase as a novel substrate of malin ubiquitination, but malin does not target glycogen phosphorylase for degradation. Glycogen phosphorylase is vital in the catabolism of cellular glycogen. The expression of glycogen phosphorylase was decreased in liver and skeletal muscle tissues from malin knockout mice. Similarly, overexpression of LD mutations of malin in HEK-293 cells decreased glycogen phosphorylase expression. In addition, overexpression of malin in HEK-293 cells increased the endogenous expression of glycogen phosphorylase. Malin interacted with and ubiquitinated glycogen phosphorylase, and the presence of malin increased the nuclear localization of glycogen phosphorylase. Malin also altered glucan-binding and phosphorylation of glycogen phosphorylase. Thus, glycogen phosphorylase is a novel substrate of malin. Further exploration into the mechanisms of this regulation will unravel insights in glycogen metabolism, physiological functions of the malin-laforin complex and will aid in betterment of LD pharmacotherapy.

Disclosures: M.S. Gentry: None. **V. Dukhande:** None. **B. Paasch:** None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

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Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: Les Turner ALS Foundation

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Brain Research Foundation

NUCATS Translational Innovation award

Title: Bringing light on to the corticospinal motor neurons: Mehr licht bitte

Authors: *P. OZDINLER;
Dept. of Neurol., Northwestern University, Feinberg Sch. of Med., CHICAGO, IL

Abstract: Corticospinal motor neurons (CSMN) have a unique executive function in the brain. They receive, integrate, translate and transmit cerebral cortex's input to spinal targets, acting as the "spokes person" for the cerebral cortex especially for the motor neuron circuitry. Even though it is the pre-motor neurons that determine CSMN function, it is CSMN that connects the brain and the spinal cord for the execution and modulation of most of the voluntary movement in humans. Therefore, understanding mechanisms that underlie their selective vulnerability and progressive degeneration is critically important for building effective treatment strategies in ALS. However, studying the details of CSMN biology has been limited due to the complexity of the cerebral cortex and the limited numbers of CSMN. However, recent developments allow their visualization, isolation and detailed investigation at different stages of the disease. We found that CSMN show varied transduction efficiency to different AAV serotypes, and that it can be retrogradely transduced by AAV2-2 in the most effective way. Most importantly, introduction of eGFP gene into exposed the cytoarchitectural details of CSMN and revealed that the apical dendrite degeneration is an early event in CSMN degeneration and lack of spines especially on apical dendrites suggest early cortical modulation defects in ALS. Generation of a novel reporter line, ¬ the UCHL1-eGFP mice¬, in which CSMN are genetically labeled now allows their detailed cellular analysis in vivo. Such studies uncovered autophagy as an intrinsic mechanism involved in CSMN apical dendrite degeneration. Our on going studies will reveal the intrinsic and extrinsic factors that are involved in CSMN vulnerability and progressive degeneration, and will help develop effective treatment strategies for ALS and related motor neuron diseases.

Disclosures: P. Ozdinler: None.

Poster

243. **Motor Neuron Disease: Mechanisms I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: C.05. Neurodegenerative Disorders and Movement Disorders

Support: NS061867 and NS068179 to RB

Title: *In vitro* characterization of RBM45, a new RNA-binding protein implicated in ALS and FTLD

Authors: *Y. L. LI[1], R. BOWSER[2];
[1]Neurol., [2]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are closely related neurodegenerative diseases with overlapping clinical, pathological and genetic characteristics. A major advancement to our understanding of the pathogenesis was the identification of mutations in the RNA-binding proteins TDP-43 and FUS in familial cases of ALS and FTLD, and the observation of cytoplasmic aggregates of these proteins in familial and sporadic ALS and FTLD. However, the motor neuron degeneration mechanism remains unknown. Our lab has recently identified a new RNA-binding protein, RBM45, with pathologic alterations in ALS and FTLD. RBM45 containing cytoplasmic inclusions were observed in both ALS and FTLD patients. RBM45 also co-localized with TDP-43 and ubiquitin inclusions in affected neurons. However, very little is known about the physiological functions and RNA-binding targets of RBM45.

We have used in vitro cultured cells to characterize the structural functions of RBM45. Domain analysis shows that RBM45 contains 3 RNA-recognition motifs, sharing similar structural elements with TDP-43 and FUS. Immunolocalization showed RBM45 is a nuclear protein, and the disruption of its nuclear localization signal (NLS) mis-localizes RBM45 to the cytoplasm. Reciprocal co-immunoprecipitation assays indicated physical interactions between RBM45 and TDP-43 and FUS. RBM45 domains responsible for these protein-protein interactions were determined using truncation analysis. We also discovered that RBM45 can self-aggregate and determined the domain required for self-aggregation. To study the RNA-mediated pathways of neurodegeneration in cultured neurons, we have developed a novel CLIP-seq (Crosslinking and Immunoprecipitation coupled RNA-seq) approach to identify the RNAs bound and regulated by RBM45. The CLIP-seq data and the genes jointly regulated by RBM45, TDP-43 and FUS will be presented.

Our results demonstrate that RBM45 is a new RNA-binding protein implicated in ALS and

FTLD. We propose that RBM45 is a nuclear protein that functions in RNA splicing and transport. Nuclear import defects and environmental stress mis-localize RBM45 to the cytoplasm, where cytoplasmic aggregation can occur. The accumulation of cytoplasmic RBM45 disrupts normal RNA processing, contributing to cell death. Future mechanistic studies of RBM45 are warranted to further define the roles of RBM45 in neurodegeneration, which will broaden therapeutic options for ALS and FTLD.

Disclosures: Y.L. Li: None. **R. Bowser:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: C.07. Developmental Disorders

Support: NIH Grant 2R25GM083270

The National Eye Institute

Title: Xenopus tadpole model for valproate-induced neurodevelopmental disorders

Authors: *E. J. JAMES, J. GU, C. RAMIREZ, A. S. KHAKHALIN, C. D. AIZENMAN; Brown Univ., Providence, RI

Abstract: Prenatal exposure to valproic acid (VPA), a commonly prescribed anticonvulsant, is known to increase the risk for autism spectrum disorder (ASD) in children. Rodents exposed to VPA in utero, have been utilized to generate VPA-induced rodent models of autism that show significant behavioral and electrophysiological phenotypes, for example cortical pyramidal neurons show significant increases in the probability of local connectivity and reductions in cell excitability. Here we develop a Xenopus tadpole model of VPA-induced neurodevelopmental disorders.

Xenopus laevis tadpoles are an easily manipulated and efficient model organism for studying neural development and function. In particular, the optic tectum has been used to study a wide variety of neurodevelopmental phenomena ranging from synaptogenesis, process outgrowth and neural circuit formation and plasticity. We tested whether exposing tadpoles to VPA during a critical neurodevelopmental time period would result in a similar neuronal phenotype to what has been described in rodents. We chronically exposed Xenopus tadpoles to VPA for 8-10 days, from stage 41, when retinal inputs innervate the tectum, to stages when the retinotectal map refines (48/49). We then recorded intrinsic excitability and connectivity of tectal neurons

utilizing whole-cell patch clamp recordings. We measured various electrophysiological properties related to synaptic transmission and membrane excitability, such as voltage-gated currents, excitatory and inhibitory synaptic transmission, and spike output. We found that chronic exposure to VPA increased the frequency and duration of spontaneous excitatory synaptic activity, increased network connectivity and excitability within the tectum, while reducing intrinsic excitability of tectal neurons compared to matched controls. These results mirror the electrophysiological findings in the valproate-treated mice studies.
Behavioral research has shown that autistic children and VPA-induced models of ASD have an increased sensitivity to sensory stimuli. Consistently, we also conducted behavioral assays on the VPA-treated tadpoles and observed an increased response to sensory stimuli, expressed as decreased habitutation and enhanced sensitization to a startle stimulus.
Xenopus tadpoles are a high-throughput model organism with well-understood neural organization useful for studying mechanisms that govern development of neural circuits. When these attributes of Xenopus are combined with our findings, we demonstrate that Xenopus tadpoles are a viable and efficient model to study the mechanisms by which VPA-induced ASD occurs.

Disclosures: E.J. James: None. **J. Gu:** None. **A.S. Khakhalin:** None. **C.D. Aizenman:** None. **C. Ramirez:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

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Topic: C.07. Developmental Disorders

Support: Grant from Ministry of Health, Labour and Welfare, H23-Kagaku-Ippan-004

JST A-STEP AS242Z01798P

KAKENHI 24500269, 24240076

Title: Attenuation of inhibitory synaptic input of hippocampal neural activity following exposure to valproic acid: A voltage-sensitive dye imaging study

Authors: *T. TOMINAGA[1], Y. TOMINAGA[1], B. JULIANDI[2], K. IGARASHI[3], K. TANEMURA[4], J. KANNO[3], K. NAKASHIMA[2];
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Grad Sch. Med. Sci, Kyushu Univ., Fukuoka, Japan; [3]Div. Cell. and Mol. Toxicol., NIHS, Tokyo, Japan; [4]Lab. Animal Reproduction, Grad Sch. Agr. Sci, Tohoku Univ., Sendai, Japan

Abstract: Valproic acid (VPA) is a first-line therapy for epilepsy. Recently, the FDA warned that children born to mothers treated with VPA are at an increased risk of developing cognitive deficits. Indeed, some studies have highlighted a relationship between these cognitive deficits and autism spectrum disorders (ASDs). It is widely accepted that disruptions to excitatory/inhibitory (E/I) balance and communication alterations between different brain areas are important factors in the etiology of ASDs. In other words, ASDs are "circuit disorders" that are more likely caused by alterations to macroscopic neural circuit function rather than alterations to specific microscopic synapses or neurons. To date, only a limited number of methods for demonstrating alterations in neuronal circuit activity have been reported. Here, we examined alterations in neural circuit activity by using single-photon wide-field voltage-sensitive dye (VSD) imaging. Previously, we found that mice born to mother's treated with VPA on embryonic days 12, 13, and 14 showed clear deficits in learning- and memory-related behavior after postnatal day 84. In the hippocampal slice preparation of VPA-treated mice, we searched for deficits in synaptic activation patterns in the perforant (PP), mossy fiber (MF), and Schaffer collateral (Sch) pathways by using VSD imaging. Hippocampal slices (thickness, 400 μm) were prepared and stained with VSD (Di-4-ANEPPS) from adult (older than 84 days) mice treated with and without VPA. Neuronal activation patterns were recorded using CMOS high-speed imager sensor technology (MiCAM Ultima, BrainVision), and single electrical stimulation was applied to the PP, MF, and Sch pathways under wide-field optical microscopy (THT-macroscope, BrainVision). The neuronal response of VPA-treated mice, as observed using VSD imaging, was not different from that of control mice; however, the overall depolarizing activities of the circuit in the presence of the GABA(A) receptor inhibitor, picrotoxin (100 μM), were significantly lower in the VPA mice following Sch and MF stimulation. In other words, the circuit activities showed reduced sensitivity to the GABA(A) receptor inhibitor after Sch and MF synaptic activation. We hypothesize that this occurs because of reduced inhibitory activation in VPA-treated mice, accompanied by reduced excitatory activation. That is, the disruption of E/I balance in the hippocampal circuit may cause the behavioral deficits.

Disclosures: T. Tominaga: None. **Y. Tominaga:** None. **B. Juliandi:** None. **K. Igarashi:** None. **K. Tanemura:** None. **J. Kanno:** None. **K. Nakashima:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

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Topic: C.07. Developmental Disorders

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Caltech Undergraduate Research Fellowship (SURF)

Amgen Scholars Program at Caltech

NSC101-2917-I-564-039

Title: Maternal infection perturbs fetal brain development through IL-6 signaling

Authors: *Z. YAN, W.-L. WU, E. Y. HSIAO, P. H. PATTERSON;
Div. of Biol., Caltech, Pasadena, CA

Abstract: Epidemiologic studies identify maternal infection as a risk factor for autism and schizophrenia in the offspring. To understand the mechanism of how maternal infection alters fetal brain development, we use a mouse model in which injection of the viral mimic poly(I:C) at mid-gestation elicits maternal immune activation (MIA). MIA offspring exhibit autistic- and schizophrenia-like behaviors as well as neuropathology characteristic of each of these disorders. Prior work demonstrated that maternal interleukin-6 (IL-6) is a key cytokine that mediates the effects of MIA on the fetus. The receptor for IL-6, IL-6Rα, is expressed in brain regions associated with pathology in autism and schizophrenia. To examine where this cytokine acts, we are studying patterns of activation of IL-6 signaling in the fetal brain. First, we demonstrate that IL-6 mRNA and protein increase in fetal brain 3 hours following maternal poly(I:C) injection. Second, we find that highly selective regions of the embryonic brain respond to MIA: phospho-STAT3 (pSTAT3) immunostaining is elevated in the hindbrain and cerebellar primordium, and laser capture micro-dissection analysis of the JAK/STAT signaling pathway yields results consistent with the immunostaining. To further examine the role of IL-6, we have generated tissue-specific IL-6Rα knockout mice.

Disclosures: Z. Yan: None. **W. Wu:** None. **E.Y. Hsiao:** None. **P.H. Patterson:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.04/S18

Topic: C.07. Developmental Disorders

Support: CIHR

Title: Modelling autism spectrum disorder through maternal immune activation in mice

Authors: I. C. XUAN[1], A. J. RAMSEY[2], *D. R. HAMPSON[3];
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Abstract: Autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder characterized by deficits in social interaction and communication as well as ritualistic repetitive behaviours. Although a small percentage of cases are caused by genetic mutation(s), the etiology of ASD is largely unknown. Epidemiological studies have suggested that children from mothers who experienced an infection during pregnancy (i.e. maternal immune activation, MIA) may be at increased risk for this disorder. This theory is supported by reports of the presence of activated astrocytes and microglia in human post-mortem brain samples and changes in cytokine levels in the sera of ASD patients. Our objective was to assess MIA in mice. We injected mouse dams (C57BL/6) with lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (Poly I:C) during mid-gestation to mimic a bacterial or viral infection, respectively. Adult male and female offspring from injected mice were analyzed separately for ASD-associated behaviours. Pathological analyses were subsequently conducted on the cerebellum by measuring the expression of microglia, astrocyte, and mature oligodendrocyte markers using immunohistochemistry/western blots. The results indicate the presence of several ASD-like behaviours in the MIA offspring, some of which are sex-dependent.

Disclosures: I.C. Xuan: None. **A.J. Ramsey:** None. **D.R. Hampson:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.05/T1

Topic: C.07. Developmental Disorders

Support: FIPE - HCPA / Project 120433

Title: Analysis of hippocampal and cortical cytokines in animal model of autism induced by prenatal exposure to valproic acid

Authors: *D. BARONIO[1], V. BAMBINI-JUNIOR[2], D. A. MENDES-DA-CRUZ[3], K. C. GROKOSKI[2], J. M. SORRENTINO[2], M. DUTRA[2], G. M. DE MELO[2], W. SAVINO[3], C. GOTTFRIED[2], R. RIESGO[1];

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Abstract: Prenatal exposure to valproic acid (VPA) in rodents has been used as a reliable animal model of autism. It is already known that alterations in the immune system of patients are common, indicating possible roles in the pathophysiology of autism. In addition, altered levels of different cytokines have been associated to cognitive impairments with relevance to this disorder. In this context, considering that most reports of cytokines studied in autism are limited to plasma and serum evaluation, we aimed in this study to evaluate the levels of different cytokines in cerebral cortex and hippocampus of mice prenatally exposed to VPA. Female BALB/c mice coming from the local breeding colony (FEPPS-Porto Alegre) were kept in a room with controlled temperature (22 ± 1 °C), water and food ad libitum, and a 12:12 light cycle (lights on at 7:00 and lights off at 19:00). They had their fertility cycle controlled, and, when on proestrus, mated overnight. In the morning, females that presented a vaginal plug would be considered pregnant. Pregnant animals received a single intraperitoneal injection of 600 mg/kg of VPA or saline (control) on gestational day 11. The offspring of both groups (VPA and control) were killed after 30 days of birth and had their cortices and hippocampi dissected. Tissues were homogenized and used to measure IFN, TNF, IL-6 and IL-17 by a mouse Th1/Th2/Th17 cytokine cytometric bead array kit and CBA software (BD Pharmingen, San Jose, CA). The VPA group presented higher amount of TNF (12 pg/mg ± 2.112, p= 0,048) in the cortex when compared to the control group (7.8 pg/mg ± 0.3469), with no alteration in the hippocampus (VPA, 12.7 pg/mg ± 3.579 and control, 8.25 pg/mg ± 3.746). The amount of cortical IL-6 increased 2 fold in VPA group (8 pg/mg ± 1.597, p= 0,0454) compared to the control (4 pg/mg ± 0.5270) and again, no hippocampal alteration was found. Cortical IL-17 level was higher in the VPA group (5 pg/mg ± 0.7984, p= 0,0148) in comparison to controls (2.6 ± 0.3835), and the hippocampal levels of this cytokine were similar in both groups (VPA, 3.470 ± 0.5264 and control, 4.137 ± 1.307). In summary, our results show a disbalance on cytokine levels in cortex of mice exposed to VPA in utero. These data should be examined carefully, since these alterations might be associated with autistic-like behaviors. It was already reported that abnormal amount of cytokines, such as IL-6 and TNF, and their receptors in rodents is related with depression and social withdraw, typical behaviors found in autism. In the future, the use of transgenic animals overexpressing these proteins in behavioral tests would clarify the influence of immunological factors on autism pathophysiology.

Disclosures: D. Baronio: None. **V. Bambini-Junior:** None. **D.A. Mendes-da-Cruz:** None. **K.C. Grokoski:** None. **J.M. Sorrentino:** None. **M. Dutra:** None. **G.M. de Melo:** None. **W. Savino:** None. **C. Gottfried:** None. **R. Riesgo:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.06/T2

Topic: C.07. Developmental Disorders

Support: EUREKA NIH R01MH084194

NIMH R0139085

Title: Early life disruption of serotonin alters microglia expression in adult rodents

Authors: A. T. GARGIULO[1], R. D. DARLING[2], Y. LU[2], M. A. LEA[1], K. L. SIMPSON[2,3], J. C. SHIH[4,5], K. CHEN[4], I. A. PAUL[3], *R. C. LIN[2,3];
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Abstract: Serotonin (5HT) has been shown to be important in neurodevelopmental processes in addition to its traditional role in adults. The 5HT system is one of the first neurotransmitter systems to appear in the brain, and it has been shown that 5HT is transiently present in non-serotonergic neurons. This early influence suggests a pervasive impact on neurodevelopment, and disruption could be long-lasting and widespread. Our laboratories and others have used a combination of techniques to better understand 5HT's role in neurodevelopment, including the use of monoamine oxidase (MAO) mutant mice and the use of rats exposed perinatally to antidepressants such as selective serotonin reuptake inhibitors (SSRIs). In these animals, we have discovered behavioral changes during sexual and social interactions, and in response to novelty and conspecifics. Anatomically, we have found a down-regulation of 5HT markers, a disruption of the interhemispheric connections between sensory and limbic cortical areas, and an up-regulation of the locus coeruleus/norepinephrine system. Importantly, most of these changes persisted in adult animals.
Because these pathological changes included altered myelin formation, oligodendrocyte morphology, and neural morphology, examination of immune responses became a next logical step. Therefore, we used immunohistochemical procedures to explore microglial expression patterns within the structures that have been shown previously to be disrupted. Male Long Evans rats were exposed intraperitoneally to either saline or to the SSRI citalopram (CTM: 20 mg/kg/day) from postnatal day 8-21. Rats and MAOA KO mice were allowed to mature to adulthood before being sacrificed. Animals were perfused and brain slices processed with CD11b or Iba1 for microglia expression, as well as various co-stains including tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH), serotonin reuptake transporter (SERT),

parvalbumin, microtubule associated protein (MAP), and myelin basic protein (MBP). Increased expression patterns were noted within both white matter and gray matter areas in both rats exposed postnatally to CTM and in MAOA KO mice. Morphological changes in microglia were also noted, suggesting increased microglial activation in experimental animals. This increased expression pattern of microglia in our animals is especially relevant given the recent PET findings of increased microglia activity in young adults with Autism Spectrum Disorder.

Disclosures: A.T. Gargiulo: None. **R.D. Darling:** None. **Y. Lu:** None. **M.A. Lea:** None. **K.L. Simpson:** None. **J.C. Shih:** None. **K. Chen:** None. **I.A. Paul:** None. **R.C. Lin:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.07/T3

Topic: C.07. Developmental Disorders

Support: IRSC MOP81369

Fondation des Étoiles

Centre des Neurosciences de Sherbrooke

Title: Group B streptococcus inflammatory response induced during gestation leads to perinatal injuries and subsequent behavioral impairments in male offspring mimicking autistic features

Authors: *J. BERGERON[1], M.-J. ALLARD[1], J. DESLAURIERS[2], S. GRIGNON[2], P. SARRET[2], L.-C. FORTIER[2], C. POYART[3], G. SÉBIRE[2];
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Abstract: Background: The prevalence of autism increases since 1970's and the causes remain unclear. Pathogen exposures during pregnancy, including group B streptococcus (GBS) are associated with perinatal brain lesions in human. Despite GBS infection is common during pregnancy, its role on the fetal brain remains unknown. However, GBS-induced inflammation plays a noxious role in placental and perinatal brain damage, and subsequent neurobehavioral impairments including autism-like traits. The autistic features affect mostly the male progeniture.

Methods: Pregnant rats were injected every 12 hours with inactivated GBS (10^9 CFU) from serotype 1a GBS, versus saline, from gestational day 19 until the end of gestation. During the juvenile period, behavioral tests assessing exploration (Open field at post-natal day (P) 15, 20, 25), sensorimotor gating (Prepulse inhibition - PPI at P35), and social interactions (at P40) were used to detect the behavioral impairments. Additionally, anxiety-like behaviors (Elevated plus maze between P105-P115) and memory impairments (novel object recognition test at P120-150) were determined in adult rats. **Results:** Decreased ability to recognize maternal odor and increased delay to reach home-bedding compartment in GBS-exposed versus unexposed pups at P9 were observed with the nest-seeking test. Behavioral studies of exploration showed decreased motor abilities only in males - not in females - exposed to GBS compared to control pups. Duration (34.0 seconds (s) ± 7.4) and number of episodes (10.5 ± 2.5) of social interactions were significantly decreased only in male exposed to GBS compared to control animals (duration: 99.6 s ± 14.1; number of episodes: 22.0 ± 4.0). PPI disclosed - only in males exposed to GBS - sensorimotor gating deficits relevant to autistic behaviors (17% of reduction, $p<0.05$). In adulthood, male rats exposed to GBS exhibited anxiety-like behaviors and showed resistance to novelty in the novel object recognition test.**Conclusion:** Inflammatory response induced by GBS during gestation affects offspring behaviors, especially male pups which developed autistic-like behaviors. This animal model will be used to uncover the mechanistic processes linking gestational inflammation to gender-specific perinatal brain injuries and their behavioral correlates, including autistic-like symptoms.

Disclosures: J. Bergeron: None. **M. Allard:** None. **J. Deslauriers:** None. **S. Grignon:** None. **P. Sarret:** None. **L. Fortier:** None. **C. Poyart:** None. **G. Sébire:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.08/T4

Topic: C.07. Developmental Disorders

Support: Howard University Faculty Seed Award U400022

Title: Effects of prenatal exposure to valproic acid on hypothalamic and limbic structures in the rat

Authors: Y. D. GILLES, N. L. POWELL, T. T. DANG, *E. K. POLSTON;
Dept. of Physiol. and Biophysics, Howard Univ. Col. of Med., WASHINGTON, DC

Abstract: Maternal use of valproic acid (VPA), a commonly prescribed anticonvulsant drug, is reportedly associated with an increased risk of autism spectrum disorders (ASD) in human offspring. In utero VPA administration has been shown to cause ASD like behavioral profiles in the rat which include heightened anxiety and disrupted social investigations. The specific neurodevelopmental abnormalities caused by VPA remain poorly understood in human and animal models. Behavioral phenotypes seen in autism suggest altered growth of brain areas involved with social affiliation and emotional state. Several hypothalamic and limbic nuclei are known to regulate these processes, supporting the premise that developmental perturbation of subcortical structures may contribute to autistic behavioral phenotypes. We have examined hypothalamic and limbic structures in an animal model for autism whereby rat fetuses are subjected to VPA early in gestation. The present study investigated the hypothesis that prenatal VPA exposure disrupts the formation of hypothalamic and limbic nuclei in the rat. To test our hypothesis, pregnant Sprague-Dawley rats were injected with VPA on gestational day 12.5 and offspring were raised to adulthood. Brains were stained with cresyl violet for analysis of nuclear volumes and cell numbers. Preliminary analyses reveal that sex differences that were evident in the hypothalamus and amygdala of control animals were not present in VPA treated offspring. Our findings suggest that one mechanism by which prenatal VPA contact results in ASD-like behavioral phenotypes may be through abnormal sexual differentiation of subcortical brain areas that mediate the social and emotional behaviors that are typically disrupted in autism.

Disclosures: Y.D. Gilles: None. **N.L. Powell:** None. **T.T. Dang:** None. **E.K. Polston:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

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Topic: C.07. Developmental Disorders

Support: KAKENHI23500516

Health Labour Sciences Research Grant

Title: Valproate application to rat fetus encourages the development of Purkinje cell dendrites and network formation in cerebellar development

Authors: *S. YOSHIDA[1], H. MURAMOTO[1], M. TANOZAKI[1], N. HOZUMI[2], Y. FUETA[3], Y. SEKINO[4];
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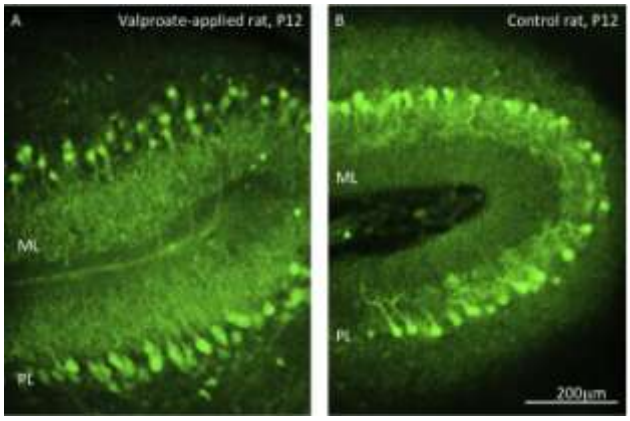
Toyohashi, Japan; [3]Univ. Occup. and Envrn. Hlth., Kitakyushu, Japan; [4]Natl. Inst. of Hlth. Sci., Tokyo, Japan

Abstract: Valproate, the popular antidepressant, is known as an inducer of autism. It has many kinds of physiological properties, including the inhibition of histone deacetylase (HDAC) and γ-amino butyric acid (GABA) transaminase. GABA plays important roles in cerebellar differentiations and functions. Recently we have developed the neurotransmitter photo assay devices for GABA, glutamate and ATP. Using these devices, we observed that GABA was much released from the glial cells in developing cerebellar external granular layer (EGL) from P3 to P7, and dropped down suddenly. ATP release to 100 μM glutamate stimulation was observed drastically at P10 in the lower molecular layer and granular layer, and decreased gradually to P14. During the first two weeks, Purkinje cells form a single layer and elongate their dendrites with synapses.

Now we investigated the effects of application of Valproate to fetus. Valproate application to embryonic day 16 p.o. increased GABA release even from early developing periods, and changed its releasing spatial pattern. ATP release was also increased from P6. In addition, Purkinje cells in the Valproate-applied rat elongate their dendrites all over the molecular layer even in P12. Figure showed anti-Calbindin D-28k antibody-stained Purkinje cells in P12. A and B is the Valproate-applied and control rat, respectively. ML: the molecular layer, PL: the Purkinje layer.

GABA would act as excitatory transmitter and/or an inducer for proliferation in developing cerebellar cortex, whereas GABA acts as inhibitory transmitter in mature brain. We suggested that Valproate application would cause precocious development of cerebellar cortex due to increase GABA release.



Disclosures: S. Yoshida: None. **H. Muramoto:** None. **M. Tanozaki:** None. **N. Hozumi:** None. **Y. Fueta:** None. **Y. Sekino:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.10/T6

Topic: C.07. Developmental Disorders

Support: Kansas City Life Science Patton Research Grant

University of Missouri Research Investment Fund

Title: Effects of prenatal stress, variable prenatal diet, and maternal genotype on offspring in an animal model of autism spectrum disorders

Authors: *K. L. JONES[1], M. WILL[2], K. FRITSCHE[2], P. HECHT[2], J. BROWNING[2], M. TILLEY[3], D. Q. BEVERSDORF[2];
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Abstract: Multiple studies have reported that prenatal stress is a possible risk factor for the development of autism. In rodents, a significant reduction in sociability is seen in adult rats exposed to prenatal stress. Genes that contribute to stress reactivity may, therefore, exacerbate prenatal stress-mediated behavioral changes in the adult offspring. Humans with the short allele of an insertion/deletion polymorphism of the SLC6A4 gene, which reduces the expression and function of the serotonin transporter (5-HTT), display increased stress reactivity. A previous rodent study found that prenatally stressed offspring of 5-HTT heterozygous (+/-) dams displayed autistic-like sociability deficits.
An additional environmental factor that may contribute to the etiology of autism is diet. One diet variable that has received recent attention is polyunsaturated fatty acids (PUFAs), particularly the decline in omega-3 PUFAs in the modern Western diet. Currently, modern Western diets are rich in omega-6 (n-6) PUFAs and deficient in omega-3 (n-3) PUFAs. A previous rodent study found that offspring exposed to a diet rich in n-6 and deficient in n-3 PUFAs during gestation and lactation displayed autistic-like sociability deficits. In contrast to n-6 PUFAs, n-3 PUFAs are known to have neuroprotective effects.
Therefore, in our study we wished to examine the role of maternal genotype, prenatal stress, and prenatal diet in mice in order to determine whether they produce autistic-like behavior in the offspring. Pregnant C57BL/6J and 5-HTT +/- dams were placed into a chronic variable stress group or a control group. Additionally, dams received one of three diets beginning 2 weeks before breeding and lasting until offspring were weaned: a control diet, a diet low in n-3 and rich in n-6 PUFAs, and a diet rich in n-3 PUFAs. We subsequently recorded the ultrasonic vocalizations of the offspring on postnatal day 8 as a measure of social communication. Beginning on postnatal day 60, the adult offspring were tested for levels of social interaction

using the 3-chamber social approach task. Anxiety was tested using the elevated-plus maze, and general activity levels were assessed using the open field. The brains of the adult offspring were then extracted and analyzed for varying levels of PUFA in whole brain tissue, and plasma was collected for immune assay analysis. Results indicated that both prenatally stressed offspring of 5-HTT +/- dams and offspring exposed to the n-3 deficient diet display autistic-like behavioral deficits. Interestingly, a perinatal diet rich in n-3 seems to ameliorate these effects in prenatally-stressed offspring of 5-HTT +/- dams.

Disclosures: K.L. Jones: None. **M. Will:** None. **K. Fritsche:** None. **P. Hecht:** None. **J. Browning:** None. **M. Tilley:** None. **D.Q. Beversdorf:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.11/T7

Topic: C.07. Developmental Disorders

Title: Effects of a prenatal vitamin d deficiency on offspring behavior in mice

Authors: *A. M. BELENCHIA[1], K. L. JONES[2], M. WILL[1], D. Q. BEVERSDORF[1], V. VIEIRA-POTTER[1], Z. FETTER[1], C. A. PETERSON[1];
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Abstract: Vitamin D deficiency/insufficiency, defined as inadequate circulating concentrations of 25-hydroxyvitamn D (25OHD), has been estimated to affect up to 75% of pregnant women in some developed nations. Multiple studies have established an associative link between low maternal 25OHD concentrations and a variety of mental health conditions, including autism spectrum disorders (ASD). The current study aimed to examine the effects of prenatal vitamin D deficiency on offspring behaviors. Female C57BL/6J mice were assigned to either a vitamin D deficient diet or a control diet two weeks prior to mating and maintained on this diet throughout pregnancy until postnatal day 7, at which point both dietary groups were switched to the control diet. We subsequently recorded the ultrasonic vocalizations of the offspring on postnatal day 8 as a measure of social communication. Beginning on postnatal day 60, the adult offspring were tested for levels of social interaction using the Crawley 3-chamber social approach task. Anxiety was tested using the elevated-plus maze, and general activity levels were assessed using the open field.

Disclosures: A.M. Belenchia: None. **K.L. Jones:** None. **M. Will:** None. **D.Q. Beversdorf:** None. **V. Vieira-Potter:** None. **Z. Fetter:** None. **C.A. Peterson:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.12/T8

Topic: C.07. Developmental Disorders

Title: Aminopeptidase P1 deficiency causes neurodevelopmental disorders in mice

Authors: *Y.-S. BAE, S. YOON, M.-S. MUN, K.-Y. PARK, W. KIM, J.-H. CHA, M.-H. KIM; Dept. of Physiol. & Biophysics, Seoul Natl. Univ. Col. of Med., Jongno-Gu, Seoul, Korea, Republic of

Abstract: Aminopeptidase Ps (APPs) catalyze the cleavage of the N-terminal amino acid residue of a peptide chain when the penultimate amino acid is proline. In mammals, there are three known isoforms of APPs (APP1-3) encoded by distinct genes (Xpnpep1-3). Many bioactive peptide hormones and neuropeptides have proline residues in their N-terminal penultimate site, and expected to be effectively degraded by the action of APPs. According to the previous report, reduced level of APP activity in humans leads to urinary excretion of imino-oligopeptides. In addition to this peptiduria, an APP-deficient human subject exhibited developmental retardation and microcephaly. However, APP isoform associated with such dysfunctions in the subject still remains unknown. In this study, we genetically disrupted APP1 function in mice and investigated the causal relationship between APP1-deficiency and developmental dysfunctions. Interestingly, urinary excretion of undigested imino-oligopeptides was observed in APP1 knockout mice, which was consistent with human subjects with reduced APP activity. In addition, APP1 deficiency in mice caused severe developmental retardation and microcephaly. These dysfunctions were associated with hippocampal pathology and abnormal synaptic transmission. These results indicate that APP1-dependent peptide metabolism is important for brain development and synaptic function.

Disclosures: Y. Bae: None. **S. Yoon:** None. **M. Mun:** None. **K. Park:** None. **W. Kim:** None. **J. Cha:** None. **M. Kim:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

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Program#/Poster#: 244.13/T9

Topic: C.07. Developmental Disorders

Support: Autism Speaks Translational Postdoc Fellowship #7587

NINDS grants R01 NS073159

NINDS grants R01NS079231

Simons Foundation SFARI #256769

Title: β-catenin transcriptionally regulates Brn2 and control early brain overgrowth

Authors: *H. BELINSON[1], J. NAKATANI[1], R. Y. BIRNBAUM[2], M. BERSHTEYN[1], B. BABINEAU[1], R. J. MCEVILLY[3], J. LONG[1], K. WILLERT[4], N. AHITUV[2], M. G. ROSENFELD[3], A. WYNSHAW-BORIS[1,5];
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Abstract: Early brain overgrowth is a hallmark phenotype of autism, but the mechanisms linking regulation of brain size with social behavioral defects are unknown. The development of the neocortex is regulated by the highly conserved wingless/Wnt developmental pathway. A key component of Wnt pathway, the Dishevelled (Dvl) family of proteins, relays Wnt signals from receptors to downstream effectors. *Dvl1*-null mice exhibited abnormal social interaction however no gross brain pathological abnormalities were seen in these mice (Lijam et al., 1997; Long et al., 2004). This suggests that redundancy of *Dvl* genes may mask subtle brain abnormalities. Thus we generated *Dvl1-/-3+/-* mice and tested the development of the neocortex in these mice. We found that Dvl mutant mice displayed early embryonic brain overgrowth associated with induced proliferation and early expansion of basal neural progenitors *in vivo* and *in vitro*. In addition, brain overgrowth was associated with expansion of deep layer neurons but not superficial layer neurons. NPC expansion was caused by deregulation of a previously uncharacterized transcriptional cascade downstream of the canonical Wnt pathway. Reduced β-catenin transcriptional activity in the $Dvl1^{-/-}3^{+/-}$ NPCs resulted in down-regulation of Brn2. This relieves the repression of Brn2 on the Tbr2 promoter, which increased Tbr2 expression and the production of basal NPCs. Moreover, Brn2-/- embryos recapitulate the brain overgrowth phenotype. Importantly, preliminary results using pharmacological induction of the Wnt canonical pathway in a short
window during embryonic development rescued the embryonic brain overgrowth phenotype. The rescue of the social behavior phenotypes is currently being tested. These results support a model that spatiotemporal regulation of the β-catenin/Brn2/Tbr2 transcriptional cascade is critical for

brain development and may contribute to the social behavioral phenotype in the *Dvl* mutant mice.

Disclosures: H. Belinson: None. **J. Nakatani:** None. **R.Y. Birnbaum:** None. **M. Bershteyn:** None. **B. Babineau:** None. **R.J. McEvilly:** None. **J. Long:** None. **K. Willert:** None. **N. Ahituv:** None. **M.G. Rosenfeld:** None. **A. Wynshaw-Boris:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.14/T10

Topic: C.07. Developmental Disorders

Support: Mizzou Advantage Project Grant

Title: Specific gene and prenatal stress interaction in the development of autism spectrum disorder

Authors: *P. HECHT[1], M. TILLEY[3], K. JONES[4,1], D. Q. BEVERSDORF[2];
[2]Radiology, Neurology, Psychology, and Thompson Ctr. for Neurdevelopmental Disorders, [1]Univ. of Missouri, Columbia, MO; [3]Central Methodist Univ., Fayette, MO; [4]Univ. of California- Davis, Davis, CA

Abstract: Our previous research has shown a significant increase in prenatal stress in mothers of children with ASD with a peak at weeks 21-32 of gestation. However, not all mothers that encounter stressful situations during pregnancy have children with ASD. It is possible that genetics may play a role in stress tolerance in the development of ASD. The serotonergic system holds particular interest in this regard. An insertion/deletion polymorphism in the promoter region of the serotonin transporter (5-HTT) gene, SLC6A4, has been associated with anxiety and stress reactivity, and some studies have suggested an association with ASD in carriers of the short allele. Additionally, the 3' polyadenylation site of the serotonin transporter has been shown to have a polymorphism that has a stronger association with panic disorder than the insertion/deletion polymorphism in the promoter. Our aim is to discover which of these stress-reactive polymorphisms found on the serotonin transporter gene may interact with environmental stressors during the pregnancy to produce a higher risk for the development of ASD. Blood was collected from families with children diagnosed with ASD for genetic testing and mothers were asked to complete questionnaires regarding their history of stress exposure during pregnancy. Early evidence suggests that the 44 base-pair deletion in the 5-HTTLPR is the critical polymorphism interacting with environmental stressors to increase the risk for ASD in the

developing child. Mothers with the 5-HTTLPR short allele have higher numbers of stressors and stressor severity during pregnancy, predominantly during the critical period of pregnancy identified in our previous work, when compared to carriers of the long allele. Furthermore, when compared to the polyadenylation polymorphism, the short allele in the 5-HTTLPR is associated with more stressors and stress severity. This study is beginning to suggest a gene and environment interaction in the development of ASD. Our study continues to show the significance of stress during gestation in the etiology of ASD particularly during weeks 21-32. More importantly, this evidence further identifies a specific gene that appears to interact with prenatal stress exposure in association with this risk. While the polyadenylation site is linked with panic disorders it does not appear to be interacting with environmental stressors to increase the risk for ASD in the child. Any marginal effect shown with the polyadenylation polymorphism may be a result of the polyadenylation site being in linkage disequilibrium with 5HTTLPR. Further analysis needs to be conducted to completely understand this gene and environment interaction.

Disclosures: P. Hecht: None. **M. Tilley:** None. **K. Jones:** None. **D.Q. Beversdorf:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.15/T11

Topic: C.07. Developmental Disorders

Support: Quinnipiac University

Title: Benzyl butyl phthalate (bbp) treatment induces alterations in estrogen responses, synaptic plasticity regulation, and neuronal development in rodents

Authors: D. DEBARTOLO[1], S. JAYATILAKA[2], E. GALLAGHER[2], M. ROSE[2], *A. J. BETZ[2], E. EMERY[2];
[1]Mol. and Cell Biol., [2]Psychology, Quinnipiac Univ., Hamden, CT

Abstract: Benzyl Butyl Phthalate (BBP) is an industrial plasticizer that has an unknown action in the central nervous system. Phthalates have recently been associated with behavioral actions that are linked to their endocrine disrupting properties. The purpose of this study was to investigate the behavioral and molecular effects of neonatal exposure to BBP. We administered BBP (2.5 and 10.0 μg/ml) in sweetened food pellets to pregnant rats until post natal day 23 until pups were weaned. We found increased serum levels of 17β-Estradiol in male offspring at PND63 suggesting BBP can invoke changes in the endocrine system. There was a significant

decrease in brain weights suggesting processes regulating brain development and function such as synaptic plasticity, neuronal growth and organizing the neural circuit are influenced by the deleterious effects phthalate exposure. Lastly, animals showed decreased freezing in tests of fear conditioning and no gross motor changes. Additionally, we found BBP altered protein levels of synaptic plasticity markers such as estrogen alpha receptors, MeCP2, UBEA3, VGLUT, and gephyrin in the amygdala, distinct hippocampal compartments and the prefrontal cortex. In a separate study, anogenital distance, a hormonally sensitive developmental measure, was decreased in both male and female offspring at PND23. Lastly, embryonic primary cortical neurons were exposed to BBP in concentration of 10nM and1000nM and immunolabled. BBP exposure in these cells altered synaptic development and the amount of inhibitory synapses. We suggest that BBP administration disrupts normal learning and social behavior, and that these effects could be related to alterations in brain development and could result in a phenotype similar to what is observed in autism spectrum disorders. These findings indicate a compelling need for evaluation of acceptable levels of exposure to phthalates present in the environment.

Disclosures: D. DeBartolo: None. **S. Jayatilaka:** None. **E. Gallagher:** None. **M. Rose:** None. **A.J. Betz:** None. **E. Emery:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.16/T12

Topic: C.07. Developmental Disorders

Title: Neurotoxic effects of pre-weaning benzyl butyl phthalate (BBP) exposure on dendritic parameters in the rat

Authors: *S. K. FOLEY[1,2], R. F. MERVIS[3,1], K. SHAKFEH[2], P. HANNA[2], S. BHASKAR[2], D. PHAM[2], D. DEBARTOLO[4], A. BETZ[4];
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Abstract: Benzyl butyl phthalate (BBP) is a widely used industrial plasticizer which, if ingested, appears to have endocrine disrupting properties and may have deleterious consequences on the developing nervous system. In the present study, pregnant Sprague-Dawley rats were fed sweetened food pellets containing 10ug/ml of BBP. This dose was continued up until post-natal day 23 at which time the rat pups were sacrificed. Pregnant control rats received sweetened food

pellets with no BBP. All brains were fixed in 10% neutral buffered formalin. Coronal blocks incorporating the parietal cortex and underlying hippocampus of 4 BBP-treated and 4 control 23 day-old male rats were Golgi stained. From coded slides camera lucida drawings of randomly selected granule cells of the dentate gyrus were prepared and evaluated for extent, complexity, and distribution of the dendritic arbor. Granule cell morphology was largely unaffected by this pre-weaning BBP exposure. Layer V pyramids, conversely, were significantly impacted by BBP. Analysis of the basilar dendritic tree showed that the BBP-exposed pups had significantly larger and more complex dendritic trees of the cortical pyramids. Dendritic spine density on the layer V pyramids was not significantly altered, but greater branching indicates that there was an overall increased synaptic presence in the layer V basilar tree. The present findings suggest that pre-weaning BBP exposure - e.g., during the period of the rodent brain growth spurt - may contribute to either additional cortical dendritic growth or diminished dendritic pruning in the course of the maturation of cortical circuits. Additional studies will determine if this is a transient or long-lasting phenomena, and if additional cell populations are also at risk from BBP exposure. Regardless, there is now evidence that environmental BBP may have deleterious consequences on neuronal development which could influence brain circuitry and, ultimately, behavior.

Disclosures: S.K. Foley: None. **R.F. Mervis:** None. **D. DeBartolo:** None. **A. Betz:** None. **K. Shakfeh:** None. **P. Hanna:** None. **S. Bhaskar:** None. **D. Pham:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.17/T13

Topic: C.07. Developmental Disorders

Support: NSERC

OGS

CFI

Title: Prostaglandin E2 alters the differentiation of neuroectodermal stem cells

Authors: *C. WONG[1], H. LI[1], D. A. CRAWFORD[1,2];
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Abstract: Background: The naturally occurring prostaglandin E_2 (PGE_2) is a bioactive lipid derived from membrane phospholipids that acts as a signalling molecule, mediating

physiological processes in early neuronal development including the neuroimmunological response and synaptic plasticity. Abnormalities in the PGE_2 signalling pathway have been linked to the neuropathology of autism. We previously showed that PGE_2 can increase the amplitude of calcium transients in growth cones of Neuro-2a cells and reduce neurite outgrowth. Studies in various non-neuronal cells have found that PGE_2 can alter cell growth, migration, and adhesion. However, the effect of PGE_2 on neuronal cell motility in the developing nervous system is poorly understood.

Objective: The goal of this study is to investigate the effect of PGE_2 on neural stem cell movement. We examined the effects PGE_2 has on the behaviour of mouse neuroectodermal stem cells (NE-4C) and its differentiation into neurons.

Methods: NE-4C stem cells were differentiated into neurons via a serum-free media protocol containing vehicle, 0.1μM, or 1μM PGE_2. Time-lapse microscopy was used to collect images over a 6-8 day differentiation period. Cell migration (final distance from original, path length, and average speed), proliferation, and the formation of neurosphere were quantified. Quantitative RT-PCR was used to measure expression of EP receptors and developmental target genes.

Results: Our study shows that undifferentiated NE-4C stem cells express all EP receptors and upon differentiation into neurons, the expression profile changes significantly. We established that *in vitro* NE-4C cells normally undergo distinct stages of differentiation that are time-specific, including proliferation followed by inward migration and aggregation, neurosphere formation, and lastly, outward migration and neurite extension. 0.1μM and 1μM PGE_2 treatment alters these stages of differentiation by varying aggregation timelines and neurosphere characteristics (i.e. size and quantity).

Conclusions: Our *in vitro* results show that PGE_2 modifies the progression of NE-4C stem cell differentiation suggesting that elevation of PGE_2 during critical periods of early neuronal development may contribute to pathologies of the central nervous system.

Disclosures: C. Wong: None. **H. Li:** None. **D.A. Crawford:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.18/T14

Topic: C.07. Developmental Disorders

Title: Prenatal domoic acid exposure as an animal model of autism: Selective disruption of male pro-social behavior

Authors: *B. D. MILLS, H. PEARCE, J. TURNER, D. ZULOAGA, J. RABER, G. LAHVIS; Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Domoic acid (DA) is an algal neurotoxin that accumulates in marine fish and shellfish. When consumed DA can cause memory loss, seizures, and death. DA is hydrophilic and thus rapidly excreted so traditional epidemiology studies examining the effects of prenatal exposure cannot be employed. Although limits on DA have been imposed, rodent experiments suggest that these limits do not protect an exposed fetus or infant from subsequent impairments. Further, animal models reveal that the effects of exposure to DA bear a striking resemblance to the repetitive behaviors, social behaviors, and neuropathology of autism spectrum disorder (ASD). These similarities, combined with indications that high prevalence of ASD has been reported for coastal communities adjacent to marine environments with DA, call for further study of the effects of exposure to this algal toxin that is transiently present in our food supply. The current study examines the effects of 1.5 mg/kg DA injected into pregnant dams at E15. In order to examine the interaction between genes and behavior, two mouse strains with differing baseline levels of social behavior and seizure susceptibility were examined (FVB and B6). After 24 hours of isolation, pups were allowed to interact with a same sex litter mate where social interaction (SI) and ultrasonic vocalizations (USV) were measured. These tests were administered at PD25 and PD35. Both human studies and animal models of autism report deficits in sensorimotor gating. In order to examine the effect of DA on sensory gating, mice were tested for potential alterations in pre-pulse inhibition (PPI).
As expected, FVB mice had greater SI scores and concurrent USVs than did B6 mice. However, despite these strain differences the effects of DA on SI and USVs were comparable. Across strains, we found that mice prenatally exposed to DA had less social interaction ($p < .01$) and fewer USVs ($p < .01$) than mice prenatally exposed to saline. A sex by group interaction ($p < .01$) revealed that the main effect of social interaction could be attributed to a decrease in social interaction in male mice exposed to DA. No sex by group interaction was found for USVs showing that unlike social interaction deficits, USV deficits extend to both male and female mice exposed to DA. Unlike the effects of DA on SI and USVs, genetic background and sex-dependent effects modulated DA-induced PPI deficits. DA-exposed male FVB mice and female B6 mice showed PPI deficits. Overall, we show that prenatal exposure to DA leads to deficits in SI, USVs, and PPI, that social measures affect males more than females and that DA may have differential effects depending on genetic susceptibility.

Disclosures: B.D. Mills: None. **H. Pearce:** None. **J. Turner:** None. **D. Zuloaga:** None. **J. Raber:** None. **G. Lahvis:** None.

Poster

244. **Developmental Disorders Associated with Prenatal Events**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 244.19/T15

Topic: C.07. Developmental Disorders

Support: NSERC

Title: Effects of prostaglandin-E2 on expression of wnt-target genes during critical period of mouse brain development

Authors: *R. BHOGAL[1], C. WONG[2], H. LI[2], D. CRAWFORD[1,2];
[1]Biol., [2]Kinesiology, York Univ., Toronto, ON, Canada

Abstract: Background: Recent evidence suggests that defects in lipid signalling pathways contribute to the pathology of autism spectrum disorder (ASD). The plasma membrane phospholipids serve as a supply of bioactive molecules such as prostaglandin E2 (PGE2) which are important for normal function of the brain. Abnormalities in lipid metabolism that alter the level of PGE2 have been linked to ASD, but the molecular mechanisms are not well understood. We have previously shown that PGE2 increases the amplitude of calcium transients in growth cone of Neuro-2a cells and induces neurite retraction. Previous studies have also shown a cooperative regulation of PGE2 signalling with developmental signalling pathways such as wingless (wnt) in non-neuronal cells.
Objectives: Our current study aims to investigate the molecular mechanisms of PGE2 interaction with the wnt signalling pathway in the developing mouse brain.
Methods: Wild-type mice (C57BL/6) were administered subcutaneously with dimethyl-Prostaglandin E2 (dmPGE2) during the critical period of development (embryonic day 11, E11). mRNA was isolated from brain tissues at two prenatal stages (E16 and E19), and one postnatal stage (day 8 after birth, P8), and investigated for quantitative expression of wnt-target genes using Custom taqman gene expression assay and confirmed with real-time PCR using SYBR Green.
Results: Following maternal exposure of PGE2, individual embryos from the litter showed significant differences in the expression of three genes: mmp7 (matrix metalloproteinase-7, involved in synapse structure and function), wnt2 (involved in neurogenesis) and Fosl1 (Fos-like antigen-1, involved in cell proliferation and differentiation). Our results show that at E16, there is a pattern of increased expression of mmp7, and decreased expression of wnt2, and at E19, a pattern of decreased expression of mmp7, and increased expression of wnt2. We also found that in normal mouse development, the expression of mmp7 and wnt2 decreases or increases throughout normal brain, respectively.
Conclusion: In this study, we found that an increase of PGE2 level during early brain development caused changes in expression levels of crucial wnt-regulated neurodevelopmental genes including mmp7, wnt2 and fosl1. Interestingly, previous findings show that mmp7 can activate mmp9, which has been found to be associated with ASD. Wnt2 is also emerging as an

autism susceptibility gene, and may play a role in language development in ASD. In summary, this in vivo study provides molecular evidence that PGE2 can alter gene expression during early development and possibly contribute to brain pathologies associated with ASD.

Disclosures: R. Bhogal: None. **C. Wong:** None. **H. Li:** None. **D. Crawford:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.01/T16

Topic: C.07. Developmental Disorders

Support: NIH R01 MH0856427

Title: Development of a versatile enrichment analysis tool reveals associations between the maternal brain and mental health disorders, including autism

Authors: *B. E. EISINGER, M. C. SAUL, T. M. DRIESSEN, S. C. GAMMIE;
Zoology, Univ. of Wisconsin-Madison, Madison, WI

Abstract: In large scale gene expression studies, enrichment of disease/disorder associated gene sets is not commonly performed. Few currently available enrichment programs support this functionality, and those that do rely on a very restricted number of mainstream disease ontologies. To make enrichment analysis possible using the existing landscape of deep and varied genetic health databases, we have developed the Modular Single-set Enrichment Test (MSET). MSET is a minimal, user friendly permutation testing script that enables researchers to assess enrichment of any conceivable gene list of interest within expression results. Using nine independently curated, unique autism gene lists extracted from gene-disease databases and recent publications, a striking consensus of enrichment was detected within gene changes measured in the lateral septum (LS) of outbred postpartum mice compared to virgin. The maternal brain has translational value to behavioral disorders such as autism because both phenomena involve dramatic changes in sociability, reactivity to fear and anxiety, and affective state. A network of 160 genes identified by MSET with links to both motherhood and autism was functionally profiled, revealing that it primarily represents developmental processes such as synaptic plasticity, neuronal morphogenesis, and differentiation. In addition to autism, the maternal LS also exhibited enrichment for genes associated with bipolar disorder, schizophrenia, ADHD, and depression. Collectively, these findings suggest that plasticity of the adult brain shapes the maternal phenotype and may form the basis of sociability in natural contexts and conditions of dysregulation. The MSET approach was validated by evaluating enrichment for a battery of

twenty-nine gene lists spanning five high profile mental health disorders and one control disease (arthritis) within three sets of expression data publicly available through NCBI's Gene Expression Omnibus. It was shown that the T-box brain protein (Tbr1) knockout mouse is a promising model for autism genetics, and that methylphenidate (Ritalin) treatment specifically affects ADHD associated genes in mice. Expression data from a murine arthritis model were also analyzed, and showed high enrichment for multiple independent arthritis gene sets.

Disclosures: B.E. Eisinger: None. **M.C. Saul:** None. **T.M. Driessen:** None. **S.C. Gammie:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.02/T17

Topic: C.07. Developmental Disorders

Support: Simons Foundation

Title: Opposing effects on structural brain volumes and cortical thickness in 16p11.2 deletion and duplication carriers

Authors: *A. Y. QURESHI[1], S. MUELLER[1], A. Z. SNYDER[2], J. P. OWEN[3], W. K. CHUNG[4], E. H. SHERR[3], R. L. BUCKNER[1,5], .. AND THE SIMONS VIP CONSORTIUM[6];
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Abstract: Rare genetic copy number variations (CNVs) contribute to neurodevelopmental disorders including autism. Here we explored the influence of the recurrent ≈ 600 kb (BP4-BP5) 16p11.2 deletion and duplication in a large cohort of individuals enrolled as part of the Simons VIP Project (Simons VIP Consortium, Neuron 2012 73: 1063-1067). Participants and controls were scanned at 3T on matched scanners at one of two locations (UCSF and CHOP). Morphometric estimates were computed using Freesurfer software implemented with a custom atlas representing the anatomy of the target cohorts. Test-retest reliability for whole brain gray matter volume, surface area, and estimated intracranial volume (eTIV) exceeded r = 0.97 for individuals scanned on multiple occasions between the two sites. Cortical thickness and most subcortical structures showed reliability ~ r = 0.80. Comparison of children (ages 8 to 16) with 16p11.2 deletions (n=22) and matched controls (n=26) yielded a consistent pattern of enlarged brain volumes. Deletion of 16p11.2 was associated with significantly enlarged eTIV, increased

gray matter volume, increased cortical thickness , and increased volume in multiple subcortical structures (e.g., amygdala and hippocampus). In contrast, in adults (ages 25 to 63) duplication of 16p11.2 (n=16) was associated with opposing effects when compared to their age-matched controls (n=32). eTIV, gray matter, cortical thickness, and subcortical volumes were all significantly reduced. To understand whether the opposing effects of deletion and duplication were due to gene dosage or the age difference, allometric scaling lines were plotted to compare eTIV (which is fixed at a young age) against brain volumes (which display longitudinal change with age). The plots were well behaved and consistent with the genetic effects having influences on brain structure that were independent of age. These results suggest that the 16p11.2 CNV has distinct, opposing effects dependent on copy number. The observation that the effects generalize across multiple cortical and subcortical structures further suggests the effect's mechanism may stem from a central developmental process that is pervasive throughout the brain.

Disclosures: A.Y. Qureshi: None. **S. Mueller:** None. **A.Z. Snyder:** None. **J.P. Owen:** None. **W.K. Chung:** None. **E.H. Sherr:** None. **R.L. Buckner:** None. **.. and the Simons VIP Consortium:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.03/T18

Topic: C.07. Developmental Disorders

Support: Simons Foundation

Title: White matter microstructure in children with 16p11.2 deletions

Authors: J. P. OWEN[1], P. BUKSHPUN[1], N. POJMAN[1], T. THIEU[1], W. CHUNG[3], E. H. SHERR[1], *P. MUKHERJEE[2], .. FOR THE SIMONS VIP CONSORTIUM[4];
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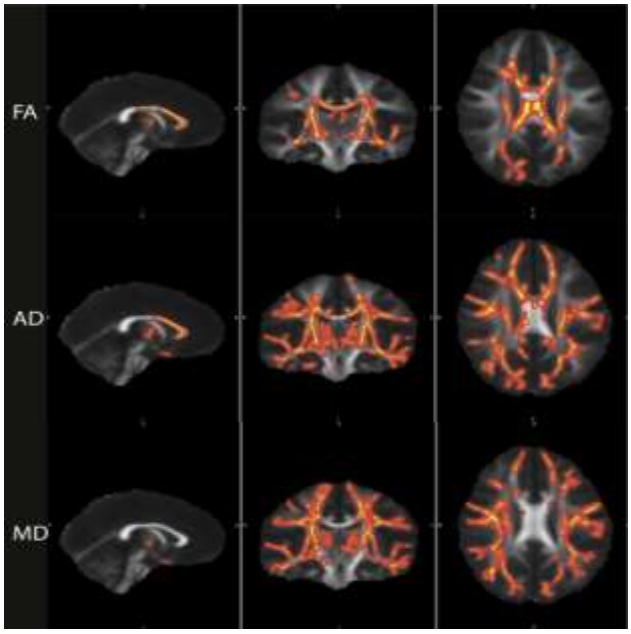
Abstract: Rare genetic copy number variations (CNVs) contribute to neurodevelopmental disorders including autism. Here we explored the influence of the recurrent ≈600 kb(BP4-BP5) 16p11.2 deletion in a large cohort of children (ages 8-16 years) enrolled as part of the Simons VIP Project [1]. We used diffusion tensor imaging (DTI) to assess changes in white matter (WM) microstructure associated with this CNV.
3T DTI of 19 child probands with 16p11.2 deletions (mean age 12.0±2.2, 10 females) and 19

controls matched for age and gender (mean age 12.7±2.5, 10 females) was acquired with 2-mm isotropic voxels and 30 directions at b=1000 s/mm^2. FSL was used to calculate fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). Nonparametric permutation testing with tract based spatial statistics (TBSS) was used to detect group differences in whole brain WM ($p<0.05$), corrected for multiple comparisons with threshold-free cluster enhancement [2].

Widespread increases in FA and AD of children with 16p11.2 deletions compared to their matched controls were detected in the genu and body of the corpus callosum, bilateral anterior and posterior corona radiata, bilateral forceps minor, bilateral forceps major, and bilateral corticospinal tracts. MD was also increased in all these areas except for the corpus callosum (Figure).

To our knowledge, this is the first demonstration of altered WM microstructure associated with 16p11.2 deletions. Abnormally reduced FA is found in many disorders; however, abnormally elevated FA is rare. One recent example, although with much less spatial extent of increased anisotropy, is Williams syndrome [3]. Correlation with animal models of these CNVs might elucidate how genes at the 16p11.2 locus govern WM microstructure. This work also motivates further research into how these gene-brain relationships affect behavior and cognitive abilities in individuals with 16p11.2 deletions.

[1] The Simons VIP Consortium. (2012) Neuron 73:1063-7. [2] Smith et al. (2006) Neuroimage 31:1487-505. [3] Hoeft et al. (2007) J Neurosci 27:11960-5.



Disclosures: J.P. Owen: None. **P. Bukshpun:** None. **N. Pojman:** None. **T. Thieu:** None. **W. Chung:** None. **E.H. Sherr:** None. **P. Mukherjee:** None. **.. for the Simons VIP Consortium:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.04/U1

Topic: C.07. Developmental Disorders

Support: Nancy Lurie Marks Family Foundation

Simons Foundation Autism Research Initiative

NIH R01 NS045193

NIH R01 NS07031

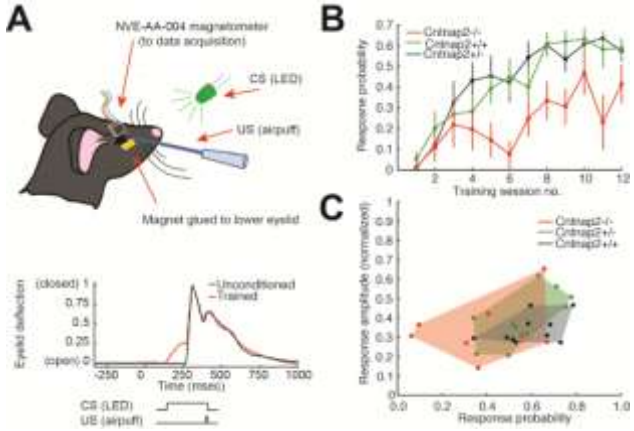
NIH F31 MH098651

Title: Phenotypic variation in mouse autism models for delay eyeblink conditioning, a form of cerebellar multisensory learning

Authors: *A. D. KLOTH[1,2], L. A. LYNCH[1,2], A. LI[1,2], R. D. JONES[2], S. G. CONNOLLY[2], M. A. BANGASH[3], O. PEÑAGARIKANO[4], P. F. WORLEY[3], D. H. GESCHWIND[4], S. S.-H. WANG[1,2];
[1]Dept. of Mol. Biol., [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD; [4]Ctr. for Autism Research, Semel Institute, and Dept. of Neurol., UCLA, Los Angeles, CA

Abstract: Persons with ASD are highly variable in their behavioral phenotype. We tested two mouse autism models for variability in delay eyeblink conditioning, a form of multisensory associative learning that requires the cerebellum. This behavior is of particular interest because (1) it is well suited to quantification and neurophysiology in head-fixed mice, (2) in humans, cerebellar injury at birth increases the risk of ASD by ~30-fold, and (3) eyeblink conditioning shows defects in both syndromic and nonsyndromic ASD (Sears et al. 1994 J Aut Dev Disord 24:737; Tobia & Woodruff-Pak 2009 Behav Neurosci 123:665).
To quantify eyeblink responses we developed a quantal analysis method. Eyelid movement amplitudes were histogrammed and separated into nonresponding and responding trials to define conditioned response (CR) probability (cf. “reliability” in synaptic plasticity literature; O’Connor, Wittenberg, & Wang 2007 Synapse 61:664) and the average response amplitude (cf. “potency”). We applied this analysis to two validated ASD models of genes highly expressed in cerebellum: the Shank3ΔC mutation (Phelan-McDermid syndrome) and the knockout of the mouse ortholog of CNTNAP2. Over 12 days of training (1 session/day), we recorded the response for each presentation of a neutral stimulus (LED flash) and a corneal airpuff.

Both Shank3(+/ΔC) and Cntnap2-/- mice and littermate wild-type controls began to generate CRs in the third training sessions. However, Shank3(+/ΔC) and Cntnap2-/- showed lower response probability at the end of training. Furthermore, CRs showed timing defects including earlier peaks and faster rise times (Kloth et al. 2012 Soc Neurosci Abstr). Blink amplitude, extinction, and reacquisition were unaltered. Both mutants showed greater variation in CR probability (coefficient of variation, CV, for Shank3(+/ΔC) = 0.28, n=14; Cntnap2-/- CV=0.61, n = 11; combined wild-type CV = 0.17, n=25). This finding suggests that within-strain genetic variation and/or environmental influences are likely to drive phenotypic variation in cerebellar developmental outcomes.



Disclosures: A.D. Kloth: None. **L.A. Lynch:** None. **A. Li:** None. **R.D. Jones:** None. **S.G. Connolly:** None. **M.A. Bangash:** None. **O. PeÑagarikano:** None. **P.F. Worley:** None. **D.H. Geschwind:** None. **S.S. Wang:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.05/U2

Topic: C.07. Developmental Disorders

Support: NSC101-2321-B-010-021

Title: Maternal valproic acid treatments induce aberrant pattern of corticostriatal system in an animal model of autism spectrum disorders

Authors: *H.-Y. KUO, F.-C. LIU;
Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Autism spectrum disorders (ASDs) are severe, heterogeneous, neurodevelopmental disorder with high prevalence around 1 in 88 individuals who share the common triad of clinical features including social impairments, communication deficits and stereotypic behaviors. Maternal valproic acid (VPA)-treated mice developed ASD-like behaviors, including social impairments and stereotypic behaviors. In the present study, we examined the effects of maternal VPA injection in the corticostriatal system with respect to the striosome-matrix compartmentation in the striatum. We found that maternal VPA injection in the time window of neurogenesis of striosomal cells resulted in reduction of mu-opioid receptor 1 (MOR1)-positive striosomes without significant changes of the calbindin-positive matrix in postnatal day (P) 14 striatum. The reduction of MOR1-positive striosomes might be due to decreases of MOR1 expression in striosomal cells. To test this possibility, we performed Western blotting. The results showed that the MOR1 protein levels were comparable between vehicle and VPA-treated striatum. Alternatively, the decreases of MOR1-positive striosomes might be resulted from failure in aggregation of striosomal cells into patches. We tested this hypothesis by pulse-labeling striosomal cells with 5-bromo-2'-deoxyuridine (BrdU), and found that unlike clusters of BrdU-labeled cells in the vehicle-treated striatum, BrdU-labeled cells tended to be scattered in the VPA-treated striatum, suggesting a defective migration of striosomal cells. Because cortical cells in deep layers share neurogenesis time windows with striosomal cells and project their axons preferentially to striosomes, we also examined the maternal VPA-treated cortex. In vehicle-treated cortex, Foxp2-positive cells were restricted to layer VI. In VPA-treated cortex, some Foxp2-positive cells were ectopically located in ER81-positive layer V. Taken together, our study indicates that maternal VPA treatments results in defective neural development of the corticostriatal system in the basal ganglia, which may underlie parts of the pathogenesis of ASDs.

Disclosures: H. Kuo: None. **F. Liu:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.06/U3

Topic: C.07. Developmental Disorders

Support: Simons Foundation VIP Project

Title: DTI and HARDI measures of anomalous auditory and language system microstructure in 16p11 deletions

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Abstract: Rare genetic copy number variations (CNVs) contribute to neurodevelopmental disorders including autism. Here we explored the influence of the recurrent 16p11.2 deletion in a large cohort of children (ages 8-16 years) enrolled as part of the Simons VIP Project (Simons VIP Consortium, Neuron 2012 73: 1063-1067). Deletion of a segment of chromosome 16 at location p11.2 has been associated with developmental disorders such as dysfunctions in language, mild to moderate intellectual disability, and autism spectrum disorders (ASD). Diffusion MR is sensitive to white matter architecture and has been previously used to detect white matter abnormalities in associated with neuropsychiatric disorders. This study uses diffusion tensor imaging (DTI) and high angular resolution diffusion imaging (DTI) to quantify alterations to the auditory and language pathways in 16p11.2 deletion.
This study included 26 healthy control (HC) children (13 male, mean age 12.1 ± 2.5 years) and 19 children (11 male, mean age 11.2 ± 2.2 yrs) with deletion of 16p11.2. Groups did not differ significantly in age or gender. MRI was performed at 3T and included a 30 diffusion gradient direction DTI and a 64 direction HARDI. DTI fiber tracking was used to identify the arcuate fasciculus (AF) and HARDI fiber tracking was used to delineate the auditory radiation. A Heschl's gyrus region of interest was identified with the Freesurfer parcellation. Voxels containing fiber tracks were used to measure tract-specific DTI parameters including fractional anisotropy (FA), mean diffusivity (MD), parallel diffusivity, and transverse diffusivity. The HARDI derived generalized fractional anisotropy (GFA) was measured in the complex white matter of the auditory radiation.
Changes in white matter microstructure were observed within the auditory and language systems. The arcuate fasciculus of deletion carriers exhibited significantly higher transverse diffusivity ($p<0.05$) and a trend toward higher mean diffusivity ($p=0.08$). The deletion group exhibited a trend toward higher mean diffusivity ($p=0.052$) in the auditory radiation. The Heschl's gyrus region contained lower FA ($p<0.01$) and higher transverse diffusivity ($p<0.02$)
The changes in diffusivity are similar to that observed in developmental disorders, particularly ASD. The present findings implicate aberrancy in WM microstructure of auditory regions in developmental and language disorders. Quantitative assessment of functionally specific regions with diffusion MR will allow us to correlate white matter microstructure with findings from electrophysical auditory and language measures (MEG) and neuropsychiatric testing.

Disclosures: J.I. Berman: F. Consulting Fees (e.g., advisory boards); McGowan Associates. **M.R. Lanza:** None. **W. Chung:** None. **T.P.L. Roberts:** None. **.. Simons VIP Consortium:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.07/U4

Topic: C.07. Developmental Disorders

Support: Simons VIP Connect FCAP

Title: A genetics based approach towards studying hemispheric dominance in magnetoencephalographic imaging (MEG-I)

Authors: *L. B. HINKLEY[1], A. FINDLAY[1], C. DALE[1], T. LUKS[1], P. BUKSHPUN[2], T. THIEU[2], N. POJMAN[2], E. MARCO[2], S. KHAN[4], K. HEIKEN[4], S. QASMIEH[4], W. CHUNG[5], T. P. L. ROBERTS[4], E. H. SHERR[3], S. S. NAGARAJAN[1], .. THE SIMONS VIP CONSORTIUM[6];
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Abstract: Rare genetic copy number variations (CNVs), specifically the recurrent ≈600 kb (BP4-BP5) 16p11.2 deletion and duplication, are known to contribute to a range of neurodevelopmental disorders, including autism. Here, we examined changes in oscillatory activity derived from magnetoencephalographic imaging (MEG-I) generated during a picture naming task in a large cohort of individuals recruited as part of the Simons VIP project (Simons VIP Consortium, Neuron 2013 73:1063-1067). We hypothesize that gene dosage in this interval is critical for the specialization of language in the left hemisphere, and that those with deletion or duplication of this locus will not develop this hemispheric dominance.
Data were acquired using 275-channel whole-head biomagnetometers (MISL; Vancouver, BC) installed at both UCSF and CHOP. During MEG recording, participants engaged in a picture naming task where an image appeared at fixation on a screen in front of the subject at the beginning of each trial (duration=1000ms), prompting the subject to respond by providing a name for the object and speaking into an optical microphone placed in front of the subject. Tomographic reconstructions of oscillatory activity across the alpha, beta, gamma and high-gamma frequency bands were locked to response onset (vocalization=0ms) and generated using an adaptive spatial filtering technique implemented in Nutmeg (nutmeg.berkeley.edu). In order to compute laterality index (LI), oscillatory activity was extracted from a pair of ROIs (frontal-temporal, temporal-parietal) in and contrasted between the left and right hemispheres.
Typically developing adults (mean age = 40.3) and children (mean age = 11.8) exhibited left-

hemisphere dominance during picture naming (LI>0.1 in 62% adults and 58% children). In contrast, in 16p11.2 child deletion carriers, 55% were right-hemispheric dominant, and 36% exhibited bilateral patterns of activity during picture naming (LI > -0.1 and < 0.1). Interestingly, in 16p11.2 adult duplication carriers only 36% were right-hemispheric dominant (LI<-0.1). A comparison between control children and 16p11.2 child deletions, LI was significantly lower in the 16p11.2 deletion group ($p<0.01$). These findings indicate that gene dosage at 16p11.2 is important for the hemispheric specialization of language.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.08/U5

Topic: C.07. Developmental Disorders

Support: Simons VIP Connect FCAP

Title: Abnormal sensory responses during semantic and facial discrimination in persons with chromosomal locus 16p11.2 deletions

Authors: *C. L. DALE[1], A. M. FINDLAY[1], L. B. HINKLEY[1], T. L. LUKS[1], P. BUKSHPUN[2], N. POJMAN[2], S. Y. KHAN[3], K. K. HEIKEN[3], S. QASMIEH[3], W. K. CHUNG[4], T. P. L. ROBERTS[3], E. H. SHERR[2], S. S. NAGARAJAN[1], .. THE SIMONS VIP CONSORTIUM[5]; [1]Dept. of Radiology and Biomed. Imaging, [2]Dept. of Neurol., UC San Francisco, SAN FRANCISCO, CA; [3]Dept. of Radiology, Children's Hosp. of Philadelphia, Philadelphia, PA; [4]Pediatrics, Columbia Univ. Med. Ctr., New York, NY; [5]The Simons Fndn., New York, NY

Abstract: Rare genetic copy number variations (CNVs) contribute to neurodevelopmental disorders, including autism (Weiss et al, 2008). We explored the influence of the recurrent ≈600 kb (BP4-BP5) 16p11.2 deletion and duplication on neural function as part of a large cohort of individuals enrolled in the Simons VIP Project (Simons VIP Consortium, Neuron 2012 73: 1063-1067). Neural activity was recorded in participants with deletion or duplication of the 16p11.2 CNV and control participants matched on age, handedness and IQ using whole-head magnetoencephalography (MEG), coregistered to individual cortical anatomy. Participants performed two discrimination tasks in the auditory and visual domain: classifying spoken words

as living or non-living and visually differentiating between face and non-face stimuli. Cortical localization of induced neural oscillations was performed using adaptive spatial filtering techniques (Dalal et al, 2011) and non-parametric statistical analyses used to compare activations between matched groups, with corrections for multiple comparisons (Singh et al 2003). During the auditory task, decreased high gamma activity (63-117 Hz) was observed in bilateral auditory cortex for 16p11.2 deletion participants relative to their control group at 100 ms post-stimulus onset. Analysis of face-specific activations revealed decreased early gamma activity (30-55 Hz) in Right Fusiform for participants with 16p11.2 deletions, as well as later decreased activity in bilateral Lingual and Middle Occipital Gyri. Analysis of evoked, broadband activity to all visual stimuli revealed deletion participants with significantly longer latencies of peak response than typically observed at 170ms following stimulus onset, as well as greater amplitude of the visual evoked field. In contrast to the patterns observed in the deletion group, duplication participants showed elevated high gamma activity of auditory cortex at 100 ms post-auditory stimulus onset, and increased late gamma band activity of Right Fusiform to face stimuli, relative to their age-matched controls. Measures of visual evoked fields in duplication participants did not differ from their age-matched controls. These results suggest pervasive abnormalities in neural oscillations induced by sensory stimuli in multiple sensory systems in participants with 16p11.2 deletions.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.09/U6

Topic: C.07. Developmental Disorders

Support: SFARI 198679

Title: Abnormal erk signaling in 16p11.2 copy number variation

Authors: *E. H. SHERR[1,2], A. FARIDAR[1], B. FREGEAU[1], P. BUKSHPUN[1], N. POJMAN[1], T. THIEU[1], .. THE SIMONS VIP CONSORTIUM[2];
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Abstract: Recurrent deletion or duplication of a ≈600 kb (BP4-BP5) 16p11.2 interval is the most common chromosomal change associated with autism. There is also evidence that dysregulation of the MAPK/ERK pathway may regulate the social deficits of autism. This locus not only contains the gene MAPK3 (ERK1), but also the gene MVP (major vault protein), a critical regulator of MAPK/ERK pathway. In this study, we evaluated whether alteration of the MAPK/ERK pathway correlates with gene dosage at the 16p11.2 locus. In patients with deletion at 16p11.2 locus, the degree of phosphorylation of ERK1/2(p-ERK1/2), as observed in freshly isolated peripheral blood mononuclear cells (PBMC), was significantly increased in comparison to controls (p-ERK/ERK: $p<0.0001$), despite the observation that protein levels for ERK1/2 were significantly decreased compared to controls ($p=0.0009$). In contrast, we found that in 16p11.2 duplication patients that the degree of p-ERK1/2 was decreased compared to controls (p-ERK/ERK: $p=0.02$). Given the prior hypothesized role for MVP in regulating ERK, tested this directly in 16p11.2 patients. We compared total MVP levels in patient cells to measures of cognitive performance, observing a statistically significant correlation between FSIQ and SRS scores and MVP protein levels ($R2=0.52$, $p=0.0016$ and $R2=0.71$ $p=0.0005$). To evaluate the role of MVP more directly in this pathway, we tested both total ERK protein levels and the degree of ERK phosphorylation in response to altering MVP levels in a cell line. We first demonstrated that we could suppress MVP translation through transient transfection of MVP siRNA into human fibroblasts. We then showed that this suppression resulted in an increase in ERK phosphorylation, without affecting total ERK protein levels. These findings demonstrate that the MAPK/ERK pathway is dysregulated in ways that are the reverse of what would be expected by gene dosage of a MAP kinase, but rather may be explained by MVP levels, secondarily regulating ERK activity. Whether these biochemical findings explain the altered behavior in these patients is subject to ongoing investigation.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

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Topic: C.07. Developmental Disorders

Support: NIH Grant R01DC010433

American Speech-Language-Hearing Foundation Research Grant for New Investigators

Title: Auditory cortex speech sound processing impairments in a rat model of autism

Authors: *C. T. ENGINEER, T. M. CENTANNI, K. W. IM, N. A. MORENO, W. A. VRANA, M. S. BORLAND, R. S. CARRAWAY, J. A. SHETAKE, K. G. RANASINGHE, J. R. RILEY, J. D. SEALE, L. G. WILSON, M. P. KILGARD;
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Abstract: Although individuals with autism are known to have significant communication problems, the neural mechanisms responsible for impaired communication are poorly understood. This study documents speech sound coding in the valproic acid (VPA) animal model of autism, and quantifies the beneficial effects of a common autism therapy, auditory training. Valproic acid is an anticonvulsant that is a known risk factor for autism in children who are prenatally exposed to it. Prenatal exposure to VPA in rats causes symptoms that mimic autism. Multiunit responses to speech sounds were collected from primary auditory cortex, anterior auditory field, ventral auditory field, and posterior auditory field in VPA exposed and control rats. Speech sounds evoke precise spatiotemporal activity patterns in the auditory cortex of control rats. Our results indicate that in utero VPA exposure severely degrades the precise spatiotemporal patterns evoked by speech sounds in anterior auditory field and ventral auditory field, but not primary auditory cortex or posterior auditory field. We next tested the hypothesis that speech sound discrimination would be impaired in VPA exposed rats. Groups of VPA exposed and control rats were trained to discriminate consonants, vowels, and tones, and were tested on their ability to generalize to novel sounds. VPA exposed rats are impaired in their ability to discriminate between some consonant sounds. Multiunit responses to speech sounds were then collected from auditory cortex of VPA exposed rats that were speech trained. Our preliminary results indicate that training enhances both speech discrimination ability and anterior auditory field responses to speech sounds. Insights derived from these studies may influence the development of new behavioral and sensory techniques to treat the communication impairments in autism that result in part from a degraded representation of speech sounds.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.11/U8

Topic: C.07. Developmental Disorders

Support: Simon's Foundation

Title: Electrophysiologic and structural markers of language impairment are seen in a genetic condition with high risk of autism spectrum disorders (ASD)

Authors: *T. P. ROBERTS[1], J. JENKINS[1], V. CHOW[1], K. HEIKEN[1], M. LANZA[1], W. CHUNG[2], J. I. BERMAN[1], .. SIMONS VIP CONSORTIUM[3];
[1]Radiology, Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Columbia Univ., New York City, NY; [3]Simon's Fndn., New York, NY

Abstract: Introduction
Rare genetic copy number variations (CNVs) contribute to neurodevelopmental disorders including autism. Here we explored the influence of the recurrent ≈600 kb(BP4-BP5) 16p11.2 deletion in a large cohort of children (ages 8-16 years) enrolled as part of the Simons VIP Project. Deletion of this segment is known to be associated with dysfunctions such as language impairment, and diagnosis of autism spectrum disorder. Previous studies using magnetoencephalography (MEG) have demonstrated delays in early auditory evoked response components (M100 and mismatch field); Diffusion tensor MRI studies have shown abnormal microstructure in white matter language pathways with the magnitude of the difference correlating with language impairment severity.
Methods
In this study we used auditory MEG and DTI in a cohort of 21 children with 16p11.2 deletions and 28 typically developing control subjects. Stimuli included sinusoidal tones to elicit M100 responses and oddball presentation of vowels to elicit MMF. DTI was performed using a Siemens 3T Verio™ system with 2mm isotropic resolution. Reconstruction of the arcuate fasciculus was performed as described in Nagae et al. 2012, using the FACT algorithm. Dependent variables were the M100 latency, MMF latency and Mean Diffusivity (MD) of the left hemisphere arcuate fasciculus.
Results
M100 delays were observed for tones of all frequencies and in both hemispheres. As in ASD, effects were most pronounced for right hemisphere responses to tones of 300Hz and 500Hz. On average a delay of 6.1ms (128.1+/-4.9 vs. 122.0+/-4.3ms) was observed in the 16p11.2 deletion cohort (approximately 50% of the delay seen in idiopathic ASD). MMF latencies were delayed 11.2ms (214.6+/-8.2 vs 203.4+/-6.6ms), again only a fraction of the effect seen (20-50ms) in ASD. Left hemisphere arcuate fasciculus MD showed elevation in 16p11.2 deletion (0.745+/-.004x10-3mm2/s vs 0.737+/-.004mm2/s) analogous to previous findings in ASD.
Conclusion
In conclusion both auditory electrophysiologic signatures and microstructural markers from DTI show abnormalities in the 16p11.2 deletion cohort that are in the same direction as those observed in idiopathic ASD, but typically approximately 50% of the magnitude. We conclude

that either 16p11.2 deletion conveys part (but not all) of the brain-level phenotype of ASD, or that this population is divided into an ASD-like cohort and a non-ASD-like cohort, with concomitant population effect reduction through averaging. Such signatures might in future form a basis for reducing such population heterogeneity.

Disclosures: T.P. Roberts: 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.; Seaside Therapeutics. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); Elekta. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Prism Clinical Imaging. **J. Jenkins:** None. **V. Chow:** None. **K. Heiken:** None. **M. Lanza:** None. **J.I. Berman:** None. **.. Simons VIP Consortium:** None. **W. Chung:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

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Topic: C.07. Developmental Disorders

Support: NIMH K23MH086785

Simons Foundation 94924

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the American Society of Maxillofacial Surgeons

Plastic Surgery Foundation

James G. Hirsch Endowed Medical Student Research Fellowship

Title: Neural correlates of language development in infants at risk for autism and infants with cranial deformities

Authors: *P. HASHIM, M. COFFMAN, C. MUKERJI, R. TILLMAN, A. NAPLES, G. RIGHI, J. TERNER, R. TRAVIESO, D. STEINBACHER, L. MAYES, J. A. PERSING, J. MCPARTLAND;
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Abstract: Background:
Language delay and difficulties in communication are characteristic features of autism spectrum disorder. Atypical lateralization of neurophysiological responses to language emerge between 6 and 12 months in high-risk infants. It remains unclear whether this atypical lateralization marks a perturbation specific to autism or a general disruption in development. Comparisons to other clinical groups are essential for establishing specificity as a biomarker for autism. Non-syndromic craniosynosyosis (NSC) is a congenital craniofacial condition also associated with language delay. Despite shared deficits, there has been no previous study comparing atypical neural development in autism and NSC.
Objective:
Our goal was to contrast electrophysiological signatures of language processing in infants at high risk for autism, infants with NSC, and infants at normal risk for autism. We compared two hypotheses: If atypically lateralized event related potentials (ERPs) to language (1) are a biomarker of autism, then only high risk infants will display the atypical response; (2) reflect general disruption of brain development, then both infants with NSC and those at high risk for autism will demonstrate atypical neural response to speech.
Methods:
The study included three groups of infants under 12 months: infants at high-risk for autism by virtue of having a sibling diagnosed with the disorder, infants with NSC, and normal-risk controls. ERPs were recorded with a 128 channel HydroCel Geodesic Sensor Net during auditory presentations of native and non-native phonemes. ERP analysis contrasted amplitudes recorded over the right and left frontal scalp to evaluate hemispheric lateralization of neural response to speech, a marker of normative language development.
Results:
Preliminary analyses focused on response to the native phoneme between the three groups at an initial positive inflection (P150). Paired samples t-test revealed hemispheric lateralization over the P150 in infants at normal risk for autism ($p = 0.043$) but not in infants at high risk for autism ($p = 0.43$) or NSC ($p = 0.33$). Ongoing analyses compare neural responses to the non-native phoneme between the three groups.
Conclusions:
This study is the first to utilize a clinical control group in order to examine the specificity of atypical ERPs to language in infants at high risk for autism. We identified atypical patterns of hemispheric lateralization of neural response to speech in both high-risk infants as well as those with NSC. These shared patterns suggest that atypical ERP responses to language may reflect a general disruption of brain development rather than a specific biomarker of autism.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

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Program#/Poster#: 245.13/U10

Topic: C.07. Developmental Disorders

Support: SFARI Explorer

Title: A new computational approach, µGWAS, identifies a novel drug target for Autism

Authors: *B. BIGIO[1], V. SONAKYA[1], M. K. TONN[3], C. NASCA[2,4], K. M. WITTKOWSKI[1]; [1]Ctr. for Clin. & Translational Sci., [2]Neuroendocrinology, The Rockefeller Univ., New York, NY; [3]RheinAhrCampus, Remagen, Germany; [4]Physiol. and Pharmacol., Univ. of Rome, Sapienza, Rome, Italy

Abstract: Autistic Spectrum Disorders (ASDs) are childhood neurodevelopment diseases with complex genetic profiles and significant lifetime neuropsychiatric comorbidity. So far, there have been no major breakthroughs in uncovering a consistent map of susceptibility loci that would pave the way in understanding ASD etiology as well as in promoting appropriate medications and behavioral therapies to prevent a genetic predisposition from becoming disease. Genome wide association analysis (GWAS) based on currently used statistical approaches require thousands of subjects and produce ambiguous results when applied to heterogeneous diseases as ASD. We extended u-statistics for multivariate data from structures among phenotype characteristics to expected linkage disequilibrium (LD) from HapMap and the spatial structure of single-nucleotide polymorphisms (SNP) within an LD block. By incorporating information about the neighborhood structure of SNPs and from HapMap about their expected LD, the novel µGWAS computational biostatistic approach increases the power of GWAS, avoids unrealistic assumptions (independence and additivity of risk factors) and reduces false positives. Applying µGWAS to childhood absence epilepsy (CAE), we identified genetic variations in Ras/Ca2+ pathways as major risk factors, consistent with a Ca2+ depolarization driven mechanism resulting in an imbalance between excitatory and inhibitory signaling in the developing brain. Based on high comorbidity of epilepsy and ASD, we hypothesized these pathways might also be involved in ASD. Indeed, a remarkable number of genes involved in the RAS/Ca2+ pathway in the two stages of the Autism Genome Project (AGP) we analyzed as independent populations, confirms that CAE and Autism share major risk factors. Our results show a strong involvement of ion channels, growth factors, and effectors of Ras. For instance, we confirmed previous results on the involvement of several genes in the regulation of synaptic plasticity. We also found consistent evidence for the involvement of receptor-type tyrosine-protein phosphatase in both populations. Remarkably, we discovered a family of genes related to

Ca2+signalingand so far not know to be involved in ASD, as targets of a drug, already approved for use in humans, that could be novel addition to the current ASD treatments. Our findings strongly supports the hypothesis that the Ras-pathways play an important role in ASD, suggesting a major role for Ca2+signaling and paving the way in developing behavioral interventions and new drugs targeting networks involved in synapse development and functions.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

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Program#/Poster#: 245.14/U11

Topic: C.07. Developmental Disorders

Support: P50 HD055751

Title: The 5HT2a receptor antagonist M100907 attenuates probabilistic reversal learning impairments in the BTBR mouse model of autism

Authors: *D. A. AMODEO[1], J. A. SWEENEY[2], M. E. RAGOZZINO[3];
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Abstract: Individuals with autism spectrum disorder (ASD) exhibit cognitive flexibility impairments that include deficits in probabilistic reversal learning. The BTBR T+ tf/J (BTBR) mouse, like ASD individuals, exhibits impairments in probabilistic reversal learning. There is increasing evidence suggesting that brain serotonin (5HT) 2a receptors are involved in cognitive flexibility. Currently, it is unknown whether treatment with a 5HT2a receptor antagonist will reduce impairments in cognitive flexibility in the BTBR mouse. The present experiment examined the effect of the 5HT2a receptor antagonist, M100907, on probabilistic reversal learning in BTBR and C57BL/6J (C57) mice. Mice were tested in a spatial discrimination task using a 80/20 probabilistic reinforcement procedure. In the spatial discrimination, mice were tested on acquisition and reversal learning across two consecutive days. Mice learned to obtain a cereal reinforcement from the “correct” spatial location (reinforced on 80% of trials) compared with the “incorrect” spatial location (reinforced on 20% of trials). The learning criterion in both phases was choosing the ‘correct’ location on 6 consecutive trials. Thirty minutes prior to the reversal learning phase, mice received injections of vehicle, 0.01, or 0.1 mg/kg of M100907. BTBR mice were not impaired on initial learning of the spatial discrimination. BTBR mice were

impaired on probabilistic reversal learning compared to that of C57 mice. M100907 treatment at both doses attenuated a reversal learning deficit in BTBR mice. M100907 treatment had no effect on reversal learning in C57 mice. M100907 0.01 mg/kg administration in BTBR mice showed a trend to reduce both perseverative and regressive errors in reversal learning. M100907 0.1 mg/kg treatment in BTBR mice attenuated a reversal learning deficit by significantly decreasing regressive errors. Thus, M100907 0.1 mg/kg treatment in BTBR mice improved the ability to reliably execute a new choice pattern during reversal learning. Because individuals with ASD exhibit cognitive flexibility deficits due to an impairment in reliably executing a new choice pattern, treatment with a 5HT2a receptor antagonist may be effective in alleviating cognitive flexibility and related features in ASD.

Disclosures: D.A. Amodeo: None. **J.A. Sweeney:** None. **M.E. Ragozzino:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 245.15/U12

Topic: C.07. Developmental Disorders

Support: Columbia Grant 7538501-GT003334

Title: Oxytocin modulates markers of the unfolded protein response in Caco2BB gut cells

Authors: *H. TAMIR[1,4,2,5], S. B. GLICKSTEIN[6], B. Y. KLEIN[2], D. L. HIRSCHBERG[3], M. M. MYERS[5], M. G. WELCH[2], M. G. WELCH[1,2];
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Abstract: The hormone oxytocin (OT) is thought to be associated with autism spectrum disorder (ASD), particularly in a subset with gut disturbance. We have demonstrated that OTR expression is developmentally regulated in rat enterocytes. Secondly, OT in combination with secretin reduces colonic inflammation in adult rats. And third, mice lacking the OTR have altered gastrointestinal motility, inflammation, macromolecular permeability, and mucosal maintenance. All of these findings suggest that understanding the function of OT in the gut could lead to an better understanding of the physiology of ASD phenotypes, and lead to new therapies. The oxytocin receptor (OTR) expression in neonatal rat enterocytes is robust from birth to weaning, but function of OTR during this period is not known. The mammalian target of rapamycin

complex1 (mTORC1) signaling is inhibited by oxytocin (OT) stimulation of Caco2BB cells (enterocytes in vitro). The unfolded protein response (UPR) protectively reduces translation during endoplasmic reticulum (ER) stress. We investigated UPR markers in OT-stimulated Caco2BB cells because of the role of mTORC1 pathway and its connection to cellular stress and ASD. We found that OT modulates several factors involved in translation and sensors of ER stress. 4E-BP1 phosphorylation (Ser65), which is known to inhibit cap-dependent translation via its rate-limiting factor eIF4E is reduced by High OT (62.5 nM). Furthermore, high OT, but not low OT (7.8 nM), increased translation elongation factor 2 (eEF2) phosphorylation (Thr56). The latter is known to inactivate eEF2. Significantly, phosphorylation of eIF2a phospho-Ser51, which inhibits eIF2a, was increased by high OT. High OT also increased PERK phosphorylation, a sensor of ER stress and a kinase of eIF2a. Finally, both high and low OT activated inositol requiring enzyme1 (IRE1), which generates the transcription factor XBP1s and induces the UPR. These findings indicate that OT modulates sensors of ER-stress and suggests that the high level of OTR expression in neonatal gut may serve a protective role during a critical post natal developmental period. These findings may contribute to understanding abnormal intracellular stress mechanisms in the autistic gut and lead to new treatments for ASD and gastrointestinal disorders.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

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Program#/Poster#: 245.16/U13

Topic: C.07. Developmental Disorders

Support: Simons Foundation

Title: Behavioral impairments in the BTBR T+tf/J mouse model of autism are improved by perinatal choline supplementation

Authors: *E. LANGLEY, M. KRYKBAEVA, J. K. BLUSZTAJN, T. J. MELLOTT; Pathology & Lab. Med., Boston Univ. Sch. of Med., Boston, MA

Abstract: Autism is a neurodevelopmental disorder involving multiple genetic and environmental risk factors and is typically characterized by deficits in social interaction, impaired communication, and restricted, repetitive behaviors. Choline is an essential nutrient for animals and humans. Prenatal and early postnatal choline supplementation has been shown to

enhance long-term cognitive performance, improve motor deficits in a mouse model of Rett syndrome, and reduce the adverse effects of neonatal alcohol exposure. We examined the effects of perinatal choline supplementation on social behavior, anxiety, and repetitive behaviors in a mouse model of autism. The BTBR T+tf/J (BTBR) inbred mouse strain exhibits many of the behavioral irregularities observed in patients with autism, including deficits in play and social approach and excessive self-grooming behavior relative to other strains. Mice from the BTBR strain and the more "sociable" control C57BL/6J (B6) strain were either fed a control or choline supplemented diet from conception through lactation and weaning of the offspring. Locomotor function, anxiety, repetitive behaviors, and social interaction were evaluated in the offspring of both strains at two time points (postnatal days 33-36 and 89-91) using the following behavioral assays: open field (OF), elevated plus maze (EPM), marble burying (MB), and three-chamber social interaction. As expected, control-diet BTBR mice displayed increased self-grooming behavior and higher locomotor activity during the OF test, a reduction in time spent in the open arms of the EMP, impaired social preference, and increased digging behavior in the MB test compared to control-diet B6 mice. Choline supplementation during development significantly reduced the frequency of digging behavior in MB test, increased the number of open arm entries and time spent in open arms in the EPM in BTBR mice, but had no effect on locomotor activity. Choline supplementation did not alter social interaction in B6 strain mice but significantly increased the time engaged in social interaction by BTBR mice. The effects of perinatal choline supplementation observed at both ages tested show that the benefits of supplementation can persist long after dietary choline returns to control levels. We are currently examining the mechanism by which choline supplementation improves these behavioral deficits. The results of this study suggest that the availability of choline during early development can prevent or reduce deficits in social behavior and anxiety in a genetic mouse model of autism, revealing a novel strategy for the treatment/prevention of autism spectrum disorders.

Disclosures: E. Langley: None. **M. Krykbaeva:** None. **J.K. Blusztajn:** None. **T.J. Mellott:** None.

Poster

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Topic: C.07. Developmental Disorders

Support: NIH Grant R01 NS25704

Simons Foundation

Title: Enhancement of α2,3-GABAA receptor signaling rescues autistic-like behaviors in a mouse model of autism

Authors: *S. HAN, C. TAI, C. J. JONES, T. SCHEUER, W. A. CATTERALL;
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Abstract: Autism spectrum disorder (ASD) may arise from increased ratio of excitatory to inhibitory neurotransmission in the brain. Many pharmacological treatments have been tested in ASD, but only limited success has been achieved. Here we report that BTBR T+tf/J (BTBR) mice, a well-established model of idiopathic autism, have reduced spontaneous GABAergic neurotransmission. Treatment with non-sedating/non-anxiolytic doses of benzodiazepines, which increase inhibitory neurotransmission through positive allosteric modulation of postsynaptic GABAA receptors, completely rescued autistic-like behaviors. Moreover, negative allosteric modulation of GABAA receptors induced autistic-like behaviors in otherwise normal C57BL/6J mice, suggesting a causal role for impaired inhibitory neurotransmission in autistic-like behaviors. The dramatic behavioral improvement after low-dose benzodiazepine treatment was subunit-specific; the α2,3-subunit-selective positive allosteric modulator L-838,417 was effective, but the α1-subunit-selective drug zolpidem exacerbated the social deficits. We propose that impaired GABAergic neurotransmission may contribute to ASD and that α2,3-subunit-selective positive GABAA receptor modulation may be an effective treatment.

Disclosures: S. Han: None. **C. Tai:** None. **C.J. Jones:** None. **T. Scheuer:** None. **W.A. Catterall:** None.

Poster

245. **"Speech, Language, and Signaling in Autism"**

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Topic: C.07. Developmental Disorders

Support: VIEP-BUAP No. FLAG/IND 13

CONACYT No. 138663 to G Flores

Title: Cerebrolysin administration restores altered anatomy and behavior in rat model of autism

Authors: *M. E. BRINGAS[1,2], O. E. APARICIO[1], A. L. SOTOMAYOR[1], S. R. ZAMUDIO[2], F. DE LA CRUZ[2], M. ATZORI[3], G. FLORES[1];
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Abstract: Prenatal valproic acid (VPA) exposure has been proposed as an animal model reproducing both behavioral and anatomical impairments of autism spectrum disorders (ASD). Some of the behavioral features of the model are lower sensitivity to pain and higher sensitivity to nonpainful stimuli, diminished acoustic prepulse inhibition, hyperresponsiveness to novel environment and decreased number of social behaviors. Among neuroanatomical changes that have been reported is a delay in eye opening related to impaired cerebral maturation; also, exposure to VPA causes abnormalities in tangential migration facial neurons and initial facial nucleus formation; finally, another important change linked to neural tube defects is the crooked tail phenotype, which also is a characteristic trait in these rats.
The neurotrophic peptide mixture Cerebrolysin (Cbl)has been reported to ameliorate abnormalities in models of aging and neurodevelopmental disorder such as Alzheimer, schizophrenia and brain injury. Only three studies about Cbl administration in autistic patients have been reported in literature with good results. For that reason, our aim in this study was to assess the effect of Cbl administration from postnatal day (PD) 5 to 21 on pups (age 21PD) and adult rats (age 70PD) prenatally VPA-exposed. We determined the effects of Cbl on different features including behavioral, neuromorphological and anatomical changes reported in the VPA animal model. Until now, we have found that Cbl administration reduces the delay in eye opening observed in VPA rats as well as attenuates the defect in crooked tail. Hyper-responsiveness to novel environment previously reported is also diminished. As far as morphology is concerned, we have found changes in dendritic morphology and density spines in prefrontal cortex, too after Cbl administration.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

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Topic: C.07. Developmental Disorders

Support: the Alberta Children's Hospital Research Institute

Title: Ketogenic diet attenuates behavioral abnormalities in the prenatal valproic acid rat model of autism spectrum disorder

Authors: *Y. AHN, S. T. NAKANISHI, R. TOBIAS, J. M. RHO, R. MYCHASIUK;
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Abstract: Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition characterized principally by impaired social interactions. Prenatal exposure of valproic acid (VPA) has been linked to ASD symptomology in humans and has been used as a model of ASD in rodents. Although the mechanisms underlying the VPA-induced ASD phenotype are not fully understood, neurometabolic changes have been implicated in other rodent ASD models. We hypothesized that the ketogenic diet (KD) - a dietary treatment that has been shown to enhance cellular bioenergetics - might improve ASD-like behaviors in VPA-exposed rats. Using a split litter design, rats exposed to VPA or saline (SAL) prenatally were administered either the KD or a standard diet (SD) from weaning until postnatal day 35 (SAL/SD; n=18, SAL/KD; n=22, VPA/SD; n=18, and VPA/KD; n=22), at which point they underwent testing in the play behaviour paradigm and the acoustic startle test, also known as the acoustic prepulse inhibition (PPI) task. Play behavior requires that each animal understands and follows a complex set of social rules and cues that can be categorized and quantified. The play behaviours analyzed for this study included evasions, complete rotations, partial rotations, and horizontal rotations. We found that prenatal VPA treatment limited both the complexity and amount of play rats engaged in, consistent with an autistic behavioral phenotype. Furthermore, we found that after KD treatment, VPA-treated rats tended to normalize their play behaviors by increasing both the diversity of play as well as the frequency of play initiations. PPI, a phenomenon in which a weak pre-stimulus decreases the startle response to an intense stimulus, provides an operational measure of sensorimotor gating (a process by which an organism filters sensory information). Our data suggest that KD treatment can increase the peak response in habituation and PPI trials. Moreover, the PPI was independently increased by both KD and VPA treatments (SAL/SD; n=17, SAL/KD; n=18, VPA/SD; n=20, and VPA/KD; n=22). These findings indicate that KD treatment may lead to improvements in ASD-like behaviors, particularly those behaviors that can be associated with higher-order social functions such as play. That said, the limbic forebrain regions more commonly associated with the PPI task may not be affected by the KD in rodent models of ASD. Based on our preliminary results, we conclude that the KD may prove to be a clinically effective intervention for ASD, one that is readily available and accessible to patients given the growing number of clinical centers worldwide that are offering this treatment.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

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INMED funds and INSERM

Neurochlore

ANR-12-RPIB-0001-01

Simons Foundation SFARI award #230267

Title: Clinical and experimental observations of bumetanide in autism

Authors: *D. C. FERRARI[1], R. TYZIO[2], R. NARDOU[1], I. KHALILOV[2], T. TSINTSADZE[1,2], C. BROUCHOUD[2], E. LEMONNIER[3], N. LOZOVAYA[2,4], N. BURNASHEV[2], Y. BEN-ARI[1,2]; [1]Neurochlore, Marseille, France; [2]INMED, INSERM U901, Aix-Marseille Univ., Marseille, France; [3]Lab. de Neurosciences de Brest, EA 4685, Brest, France; [4]INSERM U663, Univ. Paris Descartes, Paris, France

Abstract: GABAergic signals are altered in autism and GABA-acting benzodiazepines exert paradoxical effects in patients with autism suggesting that, as in epilepsies, GABA excites neurons because of elevated intracellular concentrations of chloride.
We have recently shown (Lemmonier et al, 2012) in a double-blind clinical trial on 60 children with autism (3-11 years old) that the diuretic NKCC1 chloride importer antagonist bumetanide (1mg during 3 months), that reduces intracellular chloride [Cl-]i, significantly reduced the Childhood Autism Rating Scale (CARS) and Clinical Global Impressions. Bumetanide also significantly reduced the Autism Diagnostic Observation Schedule values when the most severe cases were removed, supporting the use of bumetanide in the treatment of autism. A large multi-center European trial has been approved to be conducted soon. We have also performed in parallel an investigation on the early and late alterations of GABAergic signals and chloride homeostasis in animal models of autism (in utero valproate-treated rats (VPA), and fragile X mice). The results that confirm our working hypothesis will be presented.

Disclosures: D.C. Ferrari: A. Employment/Salary (full or part-time):; Neurochlore. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurochlore. **R. Nardou:** A. Employment/Salary (full or part-time):; Neurochlore. **Y. Ben-Ari:** A. Employment/Salary (full or part-time):; Neurochlore. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurochlore. **T. Tsintsadze:** A.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

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Topic: C.07. Developmental Disorders

Support: HRSA R40MC19926

Title: Effects of a beta-adrenergic antagonist on social and cognitive abilities in autism spectrum disorder

Authors: *R. M. ZAMZOW, B. J. FERGUSON, M. L. LEWIS, E. C. REZNICEK, A. S. RAGSDALE, S. E. CHRIST, J. P. STICHTER, D. Q. BEVERSDORF;
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social communication impairments and restricted, repetitive behaviors. Current pharmacological interventions for ASD focus primarily on psychiatric symptoms, such as agitation and obsessive behaviors. Few agents target core domains, such as social and cognitive functioning. It has been previously hypothesized that stress contributes to social and cognitive deficits in ASD. Accordingly, propranolol, a non-selective beta-adrenergic antagonist with known anxiolytic effects, may serve as a potential therapeutic agent for ASD, as it blocks the noradrenergically mediated sympathetic response system. The present ongoing study explores the effects of propranolol on social and cognitive abilities in ASD. Individuals with ASD participated in two study sessions in which they were given propranolol (40 mg) or placebo in a counterbalanced, double-blinded manner. 60 minutes following drug administration, participants performed several cognitive and social tasks. Preliminary analyses indicate a significant improvement in task performance on the General Social Outcomes Measure, an assessment of social functioning, as well as a trend for improved task performance on the Anagrams task, a measure of problem solving abilities, in the propranolol condition, as compared to the placebo condition. Continued data collection is necessary to further characterize the effects of propranolol on social and cognitive abilities in ASD. In addition, subsequent work will consider the role of baseline sympathetic nervous system activity in predicting which individuals will respond best to propranolol. Overall, these findings contribute to insight regarding potential therapeutic interventions for core symptomatology in ASD.

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Poster

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Topic: C.07. Developmental Disorders

Support: Howard Hughes Medical Institute Research Fellowship

Title: Potential contribution of pitocin and/or epidurals in labor to the development of an autism phenotype

Authors: *A. SAREEN, E. BAKER, T. WILLIAMS;
Trinity Univ., San Antonio, TX

Abstract: Autism spectrum disorders (ASDs) are a fascinating, yet perplexing group of developmental disorders because we still do not know their exact etiology. ASDs diagnoses continue to rapidly increase in number, and as of current, we know that there may be a genetic component and there is a potential for environmental influences. Since the 1970s, epidural usage has noticeably increased and previous research has shown that mothers of autistic children were more likely than mothers of non-autistic children to have received epidurals. This two-part study examines the relationship between mothers who were administered pitocin and/or an epidural during labor and the development of ASD in their offspring. All surveys were verbally administered. The survey techniques consist of interviewing mothers of ASD and non-ASD children to better understand their behavior and actions during pregnancy, including whether or not they received epidural or Pitocin. The second portion of this study involved elucidating the in vivo effects of pitocin and bupivacaube on early brain development using Drosophila melanogaster. The data from this study could yield implicative results that could influence decision-making, in terms of whether or not to use Pitocin or epidural during labor. Furthermore, this study could potentially contribute to preventative measures against this increasingly diagnosed disorder.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

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Topic: C.07. Developmental Disorders

Support: EVMS Research Enhancement Grant

Title: D-cycloserine improves sociability in the BTBR T+ Itpr3tf/J mouse model of autism spectrum disorders with altered Ras/Raf/ERK1/2 signaling

Authors: *J. A. BURKET[1], A. D. BENSON[1], A. H. TANG[2], S. I. DEUTSCH[1];
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Abstract: The genetically-inbred BTBR T+ Itpr3tf/J (BTBR) mouse is a validated model of autism spectrum disorders (ASDs). Similar to several syndromic forms of ASDs, mTOR activity may be enhanced in this mouse strain as a result of increased Ras signaling. Recently, D-cycloserine, a partial glycineB site agonist that targets the NMDA receptor, was shown to improve the sociability of the Balb/c mouse strain, another validated genetically-inbred model of ASDs. NMDA receptor activation is an important regulator of mTOR signaling activity. Given the ability of D-cycloserine to improve the sociability of the Balb/c mouse strain and the regulatory role of the NMDA receptor in mTOR signaling, we wondered if D-cycloserine would improve the impaired sociability of the BTBR mouse strain. D-Cycloserine (320 mg/kg, ip) improved measures of sociability in a standard sociability paradigm and spontaneous grooming that emerged during social interaction with an ICR stimulus mouse in the BTBR strain; however, similar effects were observed in the Swiss Webster comparator strain, raising questions about their strain-selectivity. Importantly, the profile of D-cycloserine's therapeutic effects on both measures of sociability and stereotypies is consistent with that of a desired medication for ASDs; specifically, a desired medication would not improve sociability at the expense of worsening stereotypic behaviors or vice versa. The ability of D-cycloserine to improve two prominent domains of psychopathology in ASDs in the BTBR mouse strain may be related to its ability to regulate mTOR signaling activity via stimulation of NMDA receptors.

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Poster

245. "Speech, Language, and Signaling in Autism"

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Program#/Poster#: 245.24/V3

Topic: C.07. Developmental Disorders

Support: PA Dept. of Health and Human Services

Title: Emergence of holistic processing for neural objects in autism

Authors: E. WHYTE[1], M. BEHRMANN[2], *D. ELBICH[3], N. MINSHEW[4], S. SCHERF[1]; [1]Dept. of Psychology, The Pennsylvania State Univ., University Park, PA; [2]Dept. of Psychology, Carnegie Melon Univ., Pittsburgh, PA; [3]Penn State Univ., University Park, PA; [4]Dept. of Neurol. and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Face processing deficits for individuals with autism may stem from a general perceptual bias that emphasizes featural processing at a cost to holistic processing (Behrman et al., 2006; Happe & Frith, 2006). This visuoperceputal processing bias appears to impact recognition of faces as well as other kinds of homogenous objects, including cars (Ewing et al., 2013) and novel objects (Scherf et al., 2008). In this study, we investigated whether such a visuoperceptual bias prohibits individuals with autism from learning to recognize exemplars from a class of novel objects, which only differ with respect to holistic properties. In addition, we examined whether or not this learning influenced behavioral and neural processing of faces. We tested adolescents with high functioning autism (12-18 years) in a 2-month visuoperceptual training paradigm over the course of 20 training sessions. The paradigm was designed to train participants to recognize 20 Greebles, novel objects containing two horizontal and two vertical appendages on a body, designed to invoke holistic processing demands similar to faces (Gauthier & Tarr, 1997). Importantly, the set of Greebles that were selected for training all shared the same set of appendages and only differed from one another based on the holistic organization of the features in combination with the body. Pre- and post-testing measures included the Cambridge Face Memory Task (CFMT), a composite task measuring holistic processing of untrained Greebles, as well as an fMRI task to map face-, and Greeble-related activation. In contrast to predictions from the strong version of the visuoperceptual bias argument, all 9 adolescents with autism successfully completed the training protocol and learned to recognize the 20 training Greebles. The training also lead to the emergence of holistic processing of novel Greebles in the composite task. There was no significant change in performance on the CFMT or in the size of the individually defined face-related region in the right fusiform gyrus immediately following training. These results suggest that holistic processing abilities are spared enough in adolescents with autism to learn to recognize novel objects. In addition, the widely reported deficit in face recognition abilities is not likely to be solely based on a visuoperceptual processing bias that interferes with holistic processing. These findings may have important implications for informing intervention strategies to help reduce face-processing deficits and improve adaptive functioning for individuals with autism.

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Poster

245. **"Speech, Language, and Signaling in Autism"**

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Topic: C.07. Developmental Disorders

Support: R01-DC011339-01

Title: Neural signatures of phonological working memory and grammatical processing in autism spectrum disorders

Authors: *Z. QI[1], T. PERRACHIONE[1], A. HARRIS[2], I. OSTROVSKAYA[1], S. BEACH[1], K. HALVERSON[1], A. CYR[1], K. SHER[1], M. KJELGAARD[1], J. D. E. GABRIELI[1], K. WEXLER[1], H. TAGER-FLUSBERG[2];
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Abstract: Language deficits are one of the core impairments of autism spectrum disorders (ASD). Behavioral studies have documented reduced phonological working memory capacity and impaired grammatical processing in children with ASD. The current study is the first to probe the neural characteristics of these two key language functions in children with ASD and their typically developing (TD) counterparts. Participants were all native English-speaking children and subject groups were matched on age, non-verbal IQ, and performance on standardized language tasks.
In Experiment 1, 19 children (7 ASD and 12 TD, mean age 12;1) completed a nonword repetition task during fMRI. Stimuli were pseudowords designed to match the phonological and phonotactic properties as real English words and ranged from two to five syllables in length. As predicted, the ASD group performed significantly worse than the TD group in overall accuracy (78.2% vs. 90.0%, $p < .05$). Imaging results revealed that repeating nonwords activated bilateral superior temporal gyri (STG) and left prefrontal areas in both groups, but that children with ASD showed a significantly greater task-induced activation in right middle temporal gyrus (FDR < 0.01, $q < .05$) as compared to TD children. As syllable length increased, both groups exhibited decreasing accuracy in performance ($r = 0.14$, $p < .05$) and a corresponding increased recruitment in left STG as syllabic length increased (FDR < 0.01, $q < .05$).
In Experiment 2, 22 children (10 ASD and 12 TD, mean age 12;0) completed an auditory grammaticality judgment task during fMRI. Children listened to short sentences, which were

either grammatically correct or contained morphosyntactic errors and decided if each sentence sounded correct or not. The type of errors was characteristic of those made by TD during initial language acquisition and children with language impairments for a protracted length of time (e.g. Every day he walk to school). As compared to the TD group, the ASD group performed marginally worse (91.5% vs. 97.1%, $p = .07$) on this task, and had significantly reduced task-induced activation in left inferior frontal gyrus, STG, and precentral gyrus (FDR < 0.01, $q < .05$). These preliminary results revealed hyperactivation in the right temporal lobe during the PWM task and reduced activation in the left hemisphere during grammaticality judgment in the ASD group. This works paves the way for understanding the neural characteristics underlying language deficits in children with ASD.

Disclosures: Z. Qi: None. **T. Perrachione:** None. **A. Harris:** None. **I. Ostrovskaya:** None. **S. Beach:** None. **K. Halverson:** None. **A. Cyr:** None. **K. Sher:** None. **M. Kjelgaard:** None. **J.D.E. Gabrieli:** None. **K. Wexler:** None. **H. Tager-Flusberg:** None.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.01/V5

Topic: C.07. Developmental Disorders

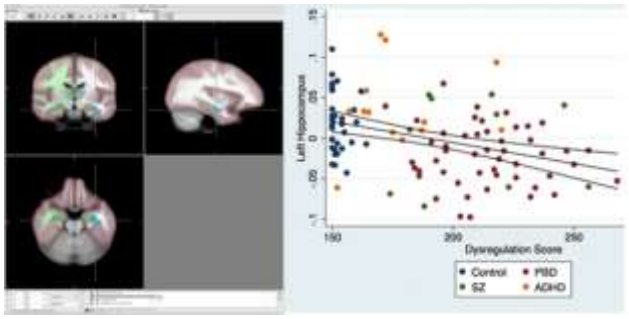
Support: NIH-NIMH R01 MH083320

Title: Child behavior checklist dysregulation profile and the brain: A VBM analysis

Authors: *D. N. KENNEDY[1], M. E. WOOD[1], E. MICK[2], J. A. FRAZIER[1];
[1]Psychiatry, [2]Quantitative Hlth. Sci., U. Massachusetts Med., WORCESTER, MA

Abstract: Background:
Behavioral dysregulation is a hallmark feature of many neurodevelopmental disorders. We sought to examine what neuroanatomic regions were related to behavioral dysregulation, as characterized by the CBCL Dysregulation Profile (CBCL-DP), using structural MRI and voxel-based morphometry.
Methods:
We identfied 120 subjects in our cohort, aged 6-18, that had a volumetric MRI and CBCL assessment. The diagnoses of these subjects included neurotypical subjects, as well as subjects with ADHD, early-onset schizophrenia, and early onset bipolar disorder. The structural images were used in a VBM analysis (FSL) in order to observe the correlation (AFNI) of gray matter density with the individualized CBCL-DP scores. These regions were further analyzed with

covariation for age and gender.
Results:
A number of regions were identified that demonstrated negative correlation between behavioral dysregulation and gray matter density, localized to the inferior lateral parietal cortex, bilaterally; left middle temporal-fusiform cortex; right parahippocampal cortex; and left hippocampus/amygdala area. After co-varying for age and gender, the left hippocampal/amygdala region demonstrated the most robust relationship.
Conclusions:
Consistent with evidence in the structural analysis literature in these disorders, the hippocampus/amygdala region is implicated for smaller gray matter density as behavioral dysregulation increases. These results promote further study of the detailed relationship of this region with the behavioral profiles in children and adolescents with neurodevelopmental psychiatric disorders.



Disclosures: D.N. Kennedy: None. **M.E. Wood:** None. **E. Mick:** None. **J.A. Frazier:** 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.; Seaside Therapeutics, Roche Pharmaceuticals.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.02/V6

Topic: C.07. Developmental Disorders

Support: DFG Grant SFB 779/TP A03

Title: Novel but also contextually rare visual stimuli activate the novelty network in children and adolescents with ADHD

Authors: *J. TEGELBECKERS[1], N. BUNZECK[2], E. DUZEL[3,4,6], B. BONATH[1,5], H.-H. FLECHTNER[1], K. KRAUEL[1];
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Abstract: Novel stimuli elicit a rapid automatic orienting response relevant for adaptive behavior in a complex and changing environment. With frequent presentation, this response declines if the stimulus has no further behavioral significance. Children with attention deficit hyperactivity disorder (ADHD) have difficulties in regulating their behavior according to situational demands. So far it is poorly understood whether altered neuronal processing of novelty contributes to these problems.
We aimed to investigate the neuronal mechanisms involved in processing novel and otherwise salient (i.e. contextually rare) stimuli in children/ adolescents with and without ADHD. Each group consisted of 19 male participants between 11-16 years. Functional brain images (3T) were recorded while participants performed a visual oddball task composed of four stimulus categories: a frequent standard picture (62.5%), a task relevant target picture (12.5%), a task irrelevant repeated rare picture (12.5%) and unique novel pictures (12.5%).
For both groups novelty related activity involved bilateral temporal and occipital regions, parahippocampal gyrus, posterior cingulate and right thalamus. However, the left superior temporal as well as inferior and middle frontal gyri showed differential effects. Specifically, processing novel stimuli was associated with stronger deactivation in healthy controls in contrast to ADHD patients. Furthermore, brain regions involved in novelty processing were also activated by contextually rare items in ADHD patients but not in healthy controls.
Our results indicate similar activity patterns to novel information in children with and without ADHD. However, the observed involvement of the novelty network in processing contextually rare items and reduced deactivations in response to task-irrelevant novelty reflect inefficient use of neuronal resources and might account for the increased distractibility in ADHD patients.

Disclosures: J. Tegelbeckers: None. **N. Bunzeck:** None. **E. Duzel:** None. **B. Bonath:** None. **H. Flechtner:** None. **K. Krauel:** None.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.03/V7

Topic: C.07. Developmental Disorders

Support: NIH Grant MH091238

Title: Atypical rich-club organization in brain connectivity as an endophenotype of attention deficit hyperactivity disorder

Authors: *M. MATTHEWS, S. RAY, J. NIGG, D. FAIR;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Attention deficit hyperactivity disorder (ADHD) is a prevalent and persistent psychiatric disorder that emerges early in childhood, with a current prevalence rate of 5.5% to 9.3% in children 4 to 17 years old. ADHD exhibits high heritability but despite numerous genes that have been identified that convey risk for ADHD, their collective risk explained is very small. The inability to clarify the genetic basis of this disorder may contribute to the heterogeneity in the population, but may also result from a lack of identified endophenotypes that better link the behavioral symptoms of the disorder with the genetic risks. Brain imaging may serve as a tool capable of identifying reliable endophenotypes with the capacity of improving our understanding of the underlying genetic component of the disorder. Based on current models of ADHD, it is likely that the cortical-subcortical interactions critical for typical development are atypical in children with this disorder, and thus may serve as a strong endophenotype. Additionally, it is likely that large-scale network properties with regard to cortico-cortical interactions are also atypical, which may be another endophenotype. To examine this possibility, we used resting state functional magnetic resonance imaging (rs-fcMRI) to measure specific neural circuits thought to be involved in ADHD, including areas such as the nucleus accumbens and the amygdala, along with graph theory analyses to identify atypical functional patterns in ADHD probands and their unaffected siblings (along with control siblings). We found no similarities or overlap in connectivity between probands with ADHD and their unaffected siblings when looking at connectivity between the nucleus accumbens or amygdala and the cortex, compared to control siblings. On the contrary, when examining network patterns such as rich-club organization, whereby highly connected nodes within a network (such as the brain) are also highly connected to each other, we observed atypical rich-club organization in functional networks of both ADHD probands and unaffected siblings. These data suggest that atypical rich-club patterns of connectivity may potentially serve as an endophenotype for ADHD. These findings provide new insight into the developmental pathobiology of ADHD and may assist in characterizing the genetic underpinnings of the disorder.

Disclosures: M. Matthews: None. **J. Nigg:** None. **D. Fair:** None. **S. Ray:** None.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.04/V8

Topic: C.07. Developmental Disorders

Support: CIHR

Title: Tracking the response inhibition network in ADHD: A joint F-MRI-DTI study

Authors: *L. K. TREMBLAY[1], M. BHAIJIWALA[1], D. MABBOTT[2], S. AMEIS[1], A. CHEVRIER[1], F. WANG[2], F. LIU[2], R. SCHACHAR[1];
[1]Psychiatry, [2]Psychology, Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Purpose : Functional MRI (F-MRI) was combined with Diffusion Tensor Imaging (DTI) to explore the integrity of the response inhibition network in childhood ADHD from a functional and structural standpoint. Response inhibition represents a key and well-studied cognitive deficit in this patient population, and an appropriate focal point in terms of elucidating the neurobiology of this complex disorder.
Methods: ADHD subjects ages 8-17 were recruited through a hospital diagnostic research clinic, which includes an interview with the patient, family and teacher, a diagnostic review and consensus by two independent clinicians, administration of parent and teacher symptom scales, extensive chart review, and psychological testing. Controls were recruited by ads and word-of-mouth. Within one study session, DTI scans were acquired along with an F-MRI study (n=8 per group), interrogating activation while participants performed the Stop Signal Task (SST). The SST is an established measure of response inhibition, probing one's ability to stop an ongoing response. F-MRI activations where ADHD differed from controls were used as seeds for DTI tractography of relevant white matter pathways. Specifically, F-MRI activation maps analyzed in AFNI were co-registered with each participant's DTI and structural MRI (T1) scans using the Automated Image Registration (AIR) and ANALYZE software. Subsequently, the FSL tools were used to perform probabilistic tractography.
Results: The F-MRI component has been analyzed in a separate study within our group, establishing that ADHD and controls differentially activate a wide network of brain regions during response inhibition, spanning from the inferior frontal gyrus (IFG), the anterior cingulate, to the cerebellum. The specific brain activations of interest, areas where ADHD clearly differed from controls during the monitoring phase of the task, were used as seed points, revealing white matter tracts of interest such as the inferior fronto-occipital fasciculus (IFOF) and the inferior longitudinal fasciculus (ILF). White matter integrity between the two groups was subsequently analyzed showing higher radial diffusivity within these tracts ($p<0.05$). A trend was seen between SST performance and white matter integrity.

Conclusions: The results of this combined structure-function study help characterize the integrity and architecture of a specific and complex cognitive network relevant in ADHD.

Disclosures: L.K. Tremblay: None. **M. Bhaijiwala:** None. **D. Mabbott:** None. **S. Ameis:** None. **A. Chevrier:** None. **F. Wang:** None. **F. Liu:** None. **R. Schachar:** None.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.05/V9

Topic: C.07. Developmental Disorders

Support: NIMH/NIH Grant 1 R01 MH091068

Title: Relationship between grit, self-control and academic achievement in ADHD

Authors: K. J. RUTLEDGE, J. F. DIXON, C. FASSBENDER, T. A. HARTANTO, E. CALFEE, *J. B. SCHWEITZER;
Dept Psychiatry & Behav Scienc, Unive Calif Davis SOM, SACRAMENTO, CA

Abstract: Attention-Deficit / Hyperactivity Disorder (ADHD) is the most common childhood behavioral disorder and persists into adulthood for a majority of individuals. The disorder is characterized by cognitive impairments and behavioral problems which have been linked to underlying neurological deficits, and individuals with ADHD typically experience downstream effects on academic achievement. Outside the ADHD literature, recent research has associated achievement with “grit,” or the personality trait of persistence and perseverance toward long term goals, demonstrating that higher grit is linked to greater achievement. This research has suggested that higher levels of grit, or persistence, can enable individuals to pursue activities that may not be very intrinsically rewarding - as may be found in the academic setting - out of motivation to achieve long-term goals. Another process relevant to academic, health and occupational success is impulsivity, or delay discounting, characterized by opting for smaller, more immediately available rewards over larger, delayed rewards. Individuals with ADHD often exhibit higher levels of delay discounting. The present study sought to examine how these variables may affect academic achievement when examined concurrently in a sample including participants with ADHD. Male and female children with ADHD (of either the inattentive subtype, IA, or combined subtype, CO) and without ADHD (TD) between the ages of 12 and 17 years completed a battery of assessments including the Short Grit Scale and others assessing academic achievement, ADHD symptoms, cognitive tempo, and delay discounting. Participant scores across measures were compared categorically between participant subtypes (TD, IA, or

CO), as well as continuously in models with other variables of interest. Results demonstrated significant subtype differences with respect to grit, such that IAs is significantly associated with lower grit scores than did TDs, and delay discounting, as COs were more impulsive than TDs. Academic achievement scores were also related to subtype, ADHD symptoms, cognitive tempo, and delay discounting. These findings point to possible new avenues to consider when examining the etiology of deficits in academic performance in ADHD, as well as potential new targets related to symptoms for treatment that may alter developmental trajectories in children with ADHD.

Disclosures: K.J. Rutledge: None. **J.F. Dixon:** None. **C. Fassbender:** None. **T.A. Hartanto:** None. **E. Calfee:** None. **J.B. Schweitzer:** None.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.06/V10

Topic: C.07. Developmental Disorders

Title: Altered connectivity in default mode and cingulo-opercular networks in children with adhd

Authors: *S. H. MOSTOFSKY[1,3], M. NEBEL[1], L. JACOBSON[1], J. WEXLER[4], B. S. CAFFO[5], J. J. PEKAR[2,3], A. D. BARBER[2,3];
[1]Neurology/Developmental Cognitive Neurol., [2]Kennedy Krieger Institute, Johns Hopkins Univ. Sch. o, Baltimore, MD; [3]Johns Hopkins Sch. of Med., Baltimore, MD; [4]Kennedy Krieger Inst., Baltimore, MD; [5]Johns Hopkins Sch. of Publ. Hlth., Baltimore, MD

Abstract: Attention Deficit Hyperactivity Disorder (ADHD) is characterized by inattentiveness, hyperactivity and impulsivity. These behavioral impairments may be associated with networks involved in sustaining task control (cingulo-opercular network: CON) and self-reflective lapses of attention (default mode network: DMN). The current study examined whether connectivity with these networks was altered in ADHD. 51 ADHD and 51 typically-developing (TD) children (ages 8-12 years) were matched for age, gender, handedness, and verbal comprehension index. All children had a five-minute resting state scan. Preprocessing included slice time correction, motion correction, co-registration, segmentation, and normalization. Nuisance variables were removed from each voxel (cerebrospinal fluid and white matter using CompCor, global mean signal, and absolute and differential motion parameters). Functional images were spatially smoothed using a 6 mm FWHM filter and then temporally filtered (bandpass 0.01-0.1Hz). 6mm

radius seeds were placed in six CON regions: bilateral dorsal anterior insula (DAI), bilateral supramarginal gyrus (SMG), and anterior cingulate cortex); and three DMN regions: medial prefrontal cortex (MPFC), posterior cingulate cortex (PCC), and lateral parietal cortex (LP). Mean time-courses were extracted and full-brain connectivity maps were created for each seed. The five CON maps were averaged to make one mean CON map and the three DMN maps were averaged to make one DMN map for each subject. Second-level t-tests were then performed to examine differential connectivity between the two groups for the CON and DMN networks. Within the DMN, a number of regions were more connected in the ADHD group, including the MPFC, bilateral LP and PCC. The left orbitofrontal cortex and left dorsolateral prefrontal cortex were also more connected with the DMN in children with ADHD. Greater connectivity with the DMN in the TD group was found in a cerebellar region and in the precuneus/superior parietal cortex. Within the CON, greater connectivity in the ADHD group was found in bilateral DAI, SMG, putamen and postcentral gyri. On the left side there was greater connectivity with the inferior frontal gyrus, inferior parietal lobule and thalamus. While prior studies have found hypo-activation or hypo-connectivity within the DMN network in ADHD, the current study finds increased within-network connectivity for both the DMN and CON. In addition, children with ADHD showed altered connectivity between DMN and other brain regions. Atypical CON and DMN connectivity may contribute to ADHD-associated impairments in attentional and behavioral control in children.

Disclosures: S.H. Mostofsky: None. **M. Nebel:** None. **L. Jacobson:** None. **J. Wexler:** None. **B.S. Caffo:** None. **J.J. Pekar:** A. Employment/Salary (full or part-time):; Philips. **A.D. Barber:** None.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.07/V11

Topic: C.07. Developmental Disorders

Title: EEG source imaging indices of attentional and inhibitory processing show association with dopamine system genes

Authors: *G. MCLOUGHLIN[1,2,3], S. MAKEIG[2], J. PALMER[2], D. BRANDEIS[4,6], M. LAUCHT[5];
[1]Schwartz Ctr. for Computat. Neurosci., UC San Diego, La Jolla, CA; [2]Swartz Ctr. for Computat. Neurosci., [3]Ctr. for Behavioral Genomics, Dept. of Psychiatry, & Inst. for Genomic Med., Univ. of California San Diego, La Jolla, CA; [4]Psychiatry and Psychotherapy for Children and

Adolescents, [5]Dept. of Child and Adolescent Psychiatry and Psychotherapy, Central Inst. of Mental Hlth., Mannheim, Germany; [6]Dept. of Child and Adolescent Psychiatry, Univ. of Zurich, Zurich, Switzerland

Abstract: Attention deficit hyperactivity disorder (ADHD) like many psychiatric disorders is heterogeneous at the symptom level, and there is most likely further heterogeneity in the etiology and pathophysiology of the disorder. Uncertainties about phenotype definition in psychiatry may have impeded the discovery of risk factors for the development of disorders. One goal for research in psychiatry is to move beyond subjective and variable diagnoses to classify disorders based on identifiable neural circuits. Dopamine system genes have been loosely linked with attention deficit hyperactivity disorder and other psychiatric disorders that have deficits in inhibitory or attentional networks. In a targeted candidate gene approach, we hypothesize that dopamine system candidate genes, specifically DRD4 and COMT, will be related to electroencephalographic (EEG) indices of inhibition and attention respectively. We collected EEG and genotype data in a sample of 200 adolescents; 53 ADHD cases and 253 controls. We measured EEG activity both at the hypothesized scalp electrodes and also at the source level using independent component analysis (ICA). ICA is a statistical blind source separation technique that has been found to be useful for separating out independent EEG signals from many cortical and non-cortical sources. No genetic association between the genetic markers and ADHD diagnosis or channel ERPs (at expected locations) was evident. However, EEG source imaging identified a strong genetic association between the risk alleles for ADHD and attenuated amplitude in source components that relate functionally to attention and inhibition ($p<.001$). This finding indicates that the inconsistent replication between dopamine genes, in particular DRD4, and ADHD may be due to heterogeneity and lack of specificity at the neurocognitive level. The association of DRD4 and COMT with EEG source markers of inhibition and attention should be explored in other disorders and the general population. EEG source imaging may improve the functional characterization of specific genetic risk factors.

Disclosures: G. McLoughlin: None. **S. Makeig:** None. **J. Palmer:** None. **D. Brandeis:** None. **M. Laucht:** None.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.08/V12

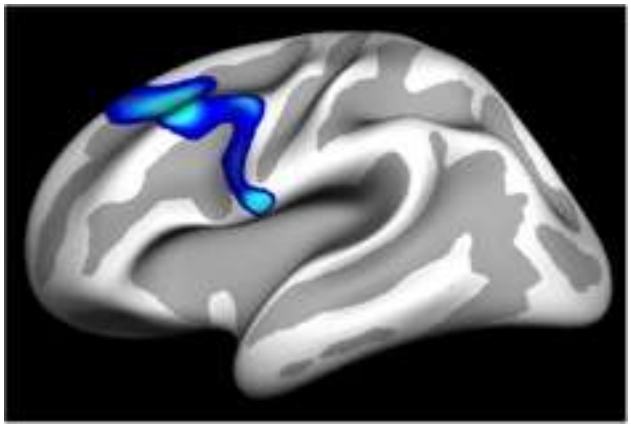
Topic: C.07. Developmental Disorders

Support: Norwegian Research Council

Title: Behavioral problems in preschool children relates to regional differences in cortical surface area

Authors: *A. BJØRNEBEKK[1,2], A. M. FJELL[2], V. MOE[3,2], K. J. Ø. HAABREKKE[3], T. SIQVELAND[3,2], K. SLINNING[3], K. B. WALHOVD[2];
[1]Dept. of Physical Med. and Rehabil., Oslo Univ. Hosp., Oslo, Norway; [2]Psychology, Univ. of Oslo, Oslo, Norway; [3]Ctr. for Child and Adolescent Mental Health, Eastern and Southern Norway (R-BUP), Natl. Network for Infant Mental Hlth. in Norway, Oslo, Norway

Abstract: Behavioral problems in children are thought to reflect a dynamic interaction between maturation and environmental factors, profoundly connected to brain development. Cortical anomalies have been linked to behavioral problems in children and recently it was shown that attention deficit hyperactivity disorder reflects a developmental delay in cerebral development of cortical thickness and surface area. In a longitudinal investigation we have followed the development of children born to mothers with drug abuse- and/or psychiatric problems and mothers without such burdens since the third trimester of pregnancy. In the current study we aimed to investigate cerebral correlates of behavioral problems in these children at 4 ½ years, thus comprising a sample exposed to pre- and postnatal risk factors for child developmental problems to various degree. 36 children underwent MR scanning and their parents completed the Child Behavior Checklist (CBCL). The relations between cortical surface area and behavioral problems were investigated throughout the cerebrum. Higher score of the CBCL Total Problem Scale were associated with reduced cortical surface area in superior frontal, caudal middle frontal, pre-central and post-central areas of the right hemisphere. Posthoc analyses are indicative that externalizing rather than internalizing behavior problems may account for the observed association. Child behavioral problems constitute an important predictor of maladjustment later in life and it is possible the observed relationship between mother assessment of behavior problems and regional reductions in surface area reflects an early susceptibility marker to later maladjustment.



Disclosures: A. Bjørnebekk: None. **A.M. Fjell:** None. **V. Moe:** None. **K.J.Ø. Haabrekke:** None. **T. Siqveland:** None. **K.B. Walhovd:** None. **K. Slinning:** None.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.09/W1

Topic: C.07. Developmental Disorders

Support: NIH Grant 4R00MH086616-03 to EAB

NIH Grant R01HD042974 to TJS

Title: Amygdalar and hippocampal volumetric and endocrine correlates of mood and anxiety in children with chromosome 22q11.2 deletion syndrome

Authors: *E. A. BEATON[1], N. Q. CUNG[2], M. H. CABARAL[2], D. D. STEPHENSON, Jr.[1], K. ANGKUSTSIRI[3], I. LECKLITER[3], T. J. SIMON[2];
[1]Dept. of Psychology, Univ. of New Orleans, New Orleans, LA; [2]MIND Inst. & Psychiatry, [3]MIND Inst. and Pediatrics, Univ. of California Davis, Sacramento, CA

Abstract: Background: Children with 22q11.2DS cope with serious medical, cognitive, and socioemotional challenges including a 20-30 percent increased risk for schizophrenia. Particular susceptibility to stress and anxiety stemming from atypical brain development, genetically-modulated temperament and cognitive impairments combined with early and ongoing traumatic experiences may contribute to the high risk of schizophrenia in this population (Beaton and Simon, 2011). We used structural magnetic resonance imaging (MRI) to measure amygdalar and hippocampal volumes in combination with daily measures of salivary cortisol (CORT) as a possible developmental marker of stress vulnerability.
Methods: As part of a larger ongoing study, participants were 7-14 year-old children with and without 22q11.2DS. They completed a series of psychological self-report and clinician-administered questionnaires measuring anxiety, and depression. High-resolution anatomical brain images were acquired using a 3T MRI scanner. Amygdalar and hippocampal volumes were calculated using a highly validated tracing methodology. Salivary cortisol was collected at five time points over two school days and one home day for a subset of children with imaging data.
Results: Children with 22q11.2DS reported higher levels of anxiety and depression versus TD children. Reduced Left amygdala volume was observed in the 22q11.2DS group versus TD children, in addition to reduced left versus right amygdala size within the 22q11.2DS group. There was no difference between left and right amygdala volumes within the TD group. Elevated anxiety predicted smaller amygdala volumes within the 22q11.2DS group but not the TD group. Hippocampal volumes did not differ between groups and hippocampal volume was not clearly

related to measures of mood or anxiety in either group. Preliminary analyses suggest that children with 22q11.2DS demonstrate a flatter diurnal rhythm of salivary CORT across days versus TD children.
Conclusions: Smaller left amygdala volumes are reported in other pediatric populations with anxiety disorders (Milham et al., 2005) and may reflect atypical brain development or stress-mediated increases in function (Schumann et al., 2004). Group differences in CORT may reflect a stress response to greater social and cognitive challenges at school experienced by children with 22q11.2DS versus TD children. The observed association between anxiety, mood, and limbic structure development is likely highly complex but may serve as a developmental risk marker in conjunction with other physiological markers of stress for later psychopathology.

Disclosures: E.A. Beaton: None. **N.Q. Cung:** None. **M.H. Cabaral:** None. **D.D. Stephenson:** None. **K. Angkustsiri:** Other; KA is sub-investigator on clinical trials sponsored by Roche, Novartis, Curemark, Forest Laboratories, and Seaside Therapeutics.. **I. Leckliter:** None. **T.J. Simon:** None.

Poster

246. **Attention Deficit Hyperactivity Disorder: Child Development**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 246.10/W2

Topic: C.07. Developmental Disorders

Support: Support from Center for International Mobility, Finland

Support from Research Unit and FUTU graduate school, Helsinki, Finland

Support from Biomedicum, Helsinki, Finland

Research grants from the Academy of Finland and from the

Title: Methylphenidate alters auditory and visual information processing in healthy volunteers

Authors: *M. KOROSTENSKAJA[1], D. KICIC[2], A. SCHAAL[1], S. KAHKONEN[2];
[1]Functional Brain Mapping and BCI Lab, Florida Hosp. For Children, Orlando, FL; [2]BioMag Laboratory, HUSLAB, Helsinki Univ. Central Hosp., Helsinki, Finland

Abstract: Introduction: Methylphenidate (MPH) is a commonly used psycho-stimulant for the treatment of attention deficit hyperactivity disorder (ADHD). Though many studies have been done to determine MPH's effectiveness in a clinical setting, little research has utilized high resolution Magnetoencephalography (MEG) combined with Electroencephalography (EEG) to

determine how MPH affects neural basis of sensory information, such as auditory and visual. Here we compare previously published study on the effect of MPH on auditory information processing by Korostenskaja et al. 2008 with the new data describing changes in visual information processing under the effect of MPH.
Methods: Neuronal activity was recorded with combined MEG/EEEG in 12 healthy subjects (F/M 5/7, age 27±5 years) after oral administration of 40 mg MPH or placebo in a randomised, double-blind, cross-over design. In auditory modality, monaural left-ear auditory stimuli were presented in an oddball paradigm with infrequent deviant tones differing in frequency and duration. We analysed both electric and magnetic N100, P200 and mismatch negativity (MMN) components. In visual modality, half-field achromatic checkerboard reversal stimuli were presented. Both electric and magnetic N75, P100, and N145 components were analyzed.
Results: During processing of auditory stimuli, MPH increased arousal levels in visual analogue scales. MPH had no effect on the dipole strength of MMN or MMN*m* in either frequency or duration deviations. MPH did, however, reduce P200 amplitudes in EEG. During processing of visual stimuli, MPH significantly increased self-reported levels of arousal and positive affect. In the MPH condition, there was a significant increase in N75 amplitude and a statistical trend for an increase in the P100 amplitude. There was a negative correlation between N75 amplitudes with the extent of changes in dizziness in VAS under the effect of MPH. The N145*m* amplitude was significantly increased after MPH administration.
Conclusions: MPH may change the neural bases of auditory information and visual information processing, such as early stimulus evaluation reflected in the P200 component as well as early and late processing of visual stimuli reflected in N75 and P145*m* components, respectively. Dopamine and noradrenaline neurotransmitter systems could be responsible for the modulation of these processes. Current results could have clinical application in the treatment of ADHD.
References: Korostenskaja M, Kicic D, Kähkönen S. Effects of methylphenidate on auditory information processing in healthy volunteers: combined EEG/MEG recording. *Psychopharmacology (Berl.)*, 197, 475-486 2008.

Disclosures: M. Korostenskaja: None. **D. Kicic:** None. **S. Kahkonen:** None. **A. Schaal:** None.

Poster

247. **Epilepsy: Glutamatergic Transmission**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 247.01/W3

Topic: C.08. Epilepsy

Support: Department of Veterans Affairs Merit Review

Title: Tomosyn dysregulation leads to aberrant glutamate release in the dentate gyrus of the hippocampus in a murine model of epileptogenesis

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Abstract: Epilepsy affects approximately 2.3 million adults and 500,000 children in the United States with 150,000 new cases being diagnosed each year. Despite the prevalence of epilepsy the aberrant molecular processes that initiate and propagate epilepsy (epileptogenesis) are unknown. Dysregulation in the release of the major excitatory neurotransmitter, glutamate, as well as in the function of several presynaptic proteins associated with neurotransmitter release have been indicated as potential causes of epileptogenesis. Tomosyn, one of the presynaptic proteins implicated, is a negative regulator of glutamate release. Here we use glutamate biosensors to measure tonic, KCl evoked, and spontaneous glutamate transients in distinct sub-regions of the hippocampus (dentate gyrus [DG], CA3, and CA1) in tomosyn wild type (Tom+/+; n = 7), tomosyn heterozygous (Tom+/-; n = 6), and tomosyn knockout (Tom-/-; n = 9) mice. We found a positive linear trend in KCl-evoked glutamate release as tomosyn levels were reduced across genotype in the DG ($t(19) = 2.81$, $p < 0.05$). A one-way ANOVA further revealed a significant difference between Tom+/+ and Tom-/- mice in KCl-evoked glutamate release in the DG (1.73±0.78 μM vs. 4.23±0.69 μM [Mean±SEM]; $F(2, 19) = 4.04$, $p < 0.05$). No differences were seen in measures of tonic or spontaneous glutamate. These results suggest that, in the DG, the dysregulation of proteins controlling glutamate synaptic transmission as well as aberrant glutamate release may contribute to epileptogenesis. Thus, the development of novel pharmacotherapies that work to modulate presynaptic proteins that regulate glutamate release may provide beneficial therapies to treat epilepsy in humans.

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Poster

247. **Epilepsy: Glutamatergic Transmission**

Location: Halls B-H

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Topic: C.08. Epilepsy

Support: RO1 NS 040337

RO1 NS 044370

UO1 NS 58204

Epilepsy Foundation

Title: AMPA receptor-mediated transmission is enhanced during status epilepticus associated with increased insertion of GluA1 subunits

Authors: *K. RAJASEKARAN, S. JOSHI, J. KAPUR;
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Abstract: We have previously demonstrated dynamic alterations in the AMPA-mediated neurotransmission associated with the expression of GluA2 subunit-lacking, calcium-permeable AMPA receptors (AMPARs) during SE. Here we extend these findings to determine SE-associated plasticity of GluA1 subunit of AMPARs and characterize underlying molecular mechanisms.

SE was induced by lithium-pilocarpine method in adult male rats. The animals were studied either 10 min (SE-10) or 60 min (SE-60) after the first Grade V behavioral seizure using electrophysiological and biochemical techniques.

AMPAR-mediated neurotransmission increased with increasing duration of SE. The frequency of action potential-independent AMPAR-mediated excitatory postsynaptic currents (mEPSCs) recorded from CA1 pyramidal neurons (PN) of SE-10 animals was similar to that recorded from naïve control animals (0.2 ± 0.04 Hz, n = 8 vs 0.4 ± 0.05 Hz, n=14), but increased in PNs of SE-60 animals (1.6 ± 0.4 Hz, n=12, $p < 0.05$). The amplitude of mEPSCs recorded from PNs from SE-10 (12 ± 0.8 pA) and SE-60 (12 ± 0.8 pA) groups were similar to that recorded from PNs of controls (11 ± 0.6 pA). The net charge transfer of AMPAR-mediated mEPSCs was significantly greater in the SE-60 group (40 ± 6 pC vs. 139 ± 30 pC; $p < 0.05$). Similar changes in the frequency or net charge transfer were not observed in the DGCs of SE animals.

The alterations in AMPAR-mediated neurotransmission were accompanied by changes in the surface expression of GluA1 subunit. The intracellular fraction of GluA1 subunit, determined using a BS^3 assay, was reduced in PNs of SE-60 animals (50 ± 10%, n=7, $p < 0.05$) but unchanged in the DGCs (104 ± 9%, n= 5, $p > 0.05$). A biotinylation assay also confirmed increased GluA1 subunit surface expression in the PNs in SE-60 animals (140 ± 12%, n=4, $p < 0.05$).

Phosphorylation of GluA1 subunit influences its surface expression, however phosphorylation of

neither S831 (118 ± 15%, n=8, p > 0.05) or S845 (117 ± 30%, n=8, p > 0.05) residues of GluA1 subunit was altered in PNs of SE-60 animals. Studies in the organotypic hippocampal slice culture revealed that treatment with NMDA (10 μM) and high extracellular potassium (10 mM KCl) increased the surface expression of GluA1 subunit (167 ± 12%, n=4). Further, treatment of animals with NMDAR open-channel blocker, MK-801 (2 mg/kg) after 10 min of continuous electrographic seizures prevented increase in GluA1 surface expression in the PNs (97 ± 10%, n=5, p > 0.05). Thus, activation of NMDARs during SE appears to trigger increased GluA1 surface expression.
Ongoing studies are focused on determining the mechanisms underlying increased GluA1 subunit surface expression during SE.

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

Poster

247. **Epilepsy: Glutamatergic Transmission**

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Topic: C.08. Epilepsy

Support: NINDS R01 NS076885

Title: Maturation of astrocytic glutamate reuptake is disrupted in a model of cortical malformation

Authors: *E. HANSON, M. ARMBRUSTER, L. ANDRESEN, D. CANTU, C. DULLA; Neurosci., Tufts Univ., Boston, MA

Abstract: Glutamate reuptake is critical to brain development and function. We have investigated the maturation of astrocytic glutamate reuptake in the developing rat cortex and hippocampus and in the malformed, hyperexcitable cortex. We hypothesize that developmental disruption of glutamate homeostasis may be epileptogenic and might drive the pathological formation of excitatory synapses in the neonatal cortex. Glutamate transporter currents were measured by voltage clamping cortical astrocytes andt photolysing MNI-glutamate near the cell body. We observed that the decay kinetics of the transporter currents were slower in immature cortex than in immature hippocampus. This difference persisted developmentally through P14. We next examined how neonatal brain insult affected glutamate transport by astrocytes during development. A cortical freeze lesion was performed on P0 rat pups, generating a focal cortical malformation. Freeze lesion animals begin to generate epileptiform network activity by P14 in region around the malformation (the paramicrogyral zone - PMZ). We examined glutamate

reuptake in the PMZ of freeze lesioned animals at P3, P7, P14, P21, and P28 and we found that at P7, PMZ astrocytes take up glutamate faster than astrocytes in control cortex. This corresponds to an increase in reactive astrocytosis in the PMZ at P7. Given that these alterations in astrocyte function occur before the onset of hyperexcitability, a remaining question is whether glutamate dysregulation during development may drive the pathological synaptogenesis associated with hyperexciteability in the malformed cortex. We hope to will expand these studies to determine whether changes in glutamate reuptake are associated with changes in synaptic and extrasynaptic glutamate concentrations as well as long-term changes in synapse number, dendritic spine structure, and cortical network organization.

Disclosures: E. Hanson: None. **M. Armbruster:** None. **L. Andresen:** None. **D. Cantu:** None. **C. Dulla:** None.

Poster

247. **Epilepsy: Glutamatergic Transmission**

Location: Halls B-H

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Program#/Poster#: 247.04/W6

Topic: C.08. Epilepsy

Title: Head-to head comparison between mGlu1 and mGlu5 receptor enhancement in the chronic treatment of absence epilepsy in WAG/Rij rats

Authors: *V. D'AMORE[1], I. SANTOLINI[1], L. LIONETTO[2], C. M. VAN RIJN[3], M. SIMMACO[2], P. J. CONN[4], S. GATTI[5], F. NICOLETTI[1,6], G. VAN LUIJTELAAR[3], R. T. NGOMBA[1];
[1]Neurosci., I.R.C.C.S., Neuromed, Pozzilli, Italy; [2]Advanced Mol. Diagnos., St. Andrea Hosp., Rome, Italy; [3]Donders Inst. for Brain, Cognition and Behaviour., Radboud Univ., Netherlands; [4]Dept. of Pharmacol, Vanderbilt Univ., Nashville, TN; [5]C.N.S. Res. F. Hoffman LaRoche Ltd, Basel, Switzerland; [6]Univ. Sapienza, Dept. of Physiol. and Pharmacol., Rome, Italy

Abstract: Spike-wave discharges (SWDs), the electroclinical hallmark of clinical absence epilepsy, are generated within cortico-thalamo-cortical circuit. mGluRs located within this network are potential targets for SWD modulatory drugs. Symptomatic WAG/Rij rats endowed with spontaneous occurring absence seizures showed that the acute administration of positive allosteric modulators (PAMs) of mGlu1 and mGlu5 receptors (RO0711401 and VU0360172, respectively) reduced the incidence of SWDs dose dependently without affecting motor behaviour (Ngomba et al., 2011 & D'Amore et al 2012). As a follow up of these previous studies it was investigated whether tolerance during a 10 day chronic treatment occurred and whether the

sensitivity for the drugs changed after treatment. mGlu receptor expression by immunoblotting and brain concentrations of both compound during the treatment were additionally determined. The mGlu5 receptor PAM, VU0360172 (3 mg/kg, s.c., daily for 10 days) reduced the incidence of SWDs without signs of tolerance and without affecting motor behaviour during chronic administration. In contrast, tolerance developed after 2 days of treatment with the mGlu1 receptor PAM, RO0711401 (10 mg/kg, s.c., daily for 10 days). Western blot analysis data showed a down regulation of both mGlu1 and mGlu5 receptors in the cerebral cortex and thalamus in response to RO0711401 administration, whereas only the mGlu5 receptor was down-regulated in response to VU0360172 treatment. Brain levels of RO0711401 decreased with time during treatment, whereas VU0360172 levels remained constant. These data confirm the efficacy of mGlu1 and mGlu5 PAMs in the treatment of absence epilepsy, and suggest that tolerance may develop to chronic activation of mGlu1 receptors as a result of pharmacodynamic and pharmacokinetic mechanisms.

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Poster

247. **Epilepsy: Glutamatergic Transmission**

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Topic: C.08. Epilepsy

Support: Melbourne International Research Scholarship

Title: Developmental expression profile of transmembrane AMPA receptor regulatory proteins and AMPA receptor subunits in Genetic Absence Epilepsy Rats from Strasbourg

Authors: *P. M. CASILLAS-ESPINOSA, R. BARMANRAY, T. J. O'BRIEN, K. L. POWELL;
Med., Melbourne Brain Centre. Royal Melbourne Hospital. The Univ. of Melbourne, Parkville, Australia

Abstract: Objective: Absence epilepsy is characterized by a temporary loss of awareness and spike-and-wake discharges on the EEG. Studies in the well validated Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model have identified that absence seizures arise due to abnormal hypersynchronous activity within the thalamocortical circuit. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptors (AMPARs) mediate most of fast excitatory synaptic

neurotransmission. Transmembrane AMPAR regulatory proteins (TARPs), stargazin (γ2), γ3, γ4, γ5, γ7 and γ8, are a family of proteins that regulate AMPARs trafficking, expression and biophysical properties. Stargazin has been shown to be increased in the somatosensory cortex (SCx) of GAERS compared to non-epileptic control (NEC) rats. In this study we investigate the expression of TARPs and AMPARs in different developmental ages, 7 day (pre-epileptic), 7 weeks (early epileptic) and 22 weeks (epileptic) to determine if expression changes are causative or a consequence of the epilepsy. Methods: Male GAERS (n=10) and NECs (n=10), aged 5 and 20 week underwent surgery to implant EEG recording electrodes for confirmation of the epileptic phenotype. After a one week recovery, 2 x 4hr EEG recordings were acquired over a week after which time the animals were culled, the brains were rapidly extracted and the SCx, motor cortex (MCx) and thalamus were dissected, snap-frozen over liquid nitrogen and stored at −80 °C. GAERS (n=10) and NECs (n=10) 7 day old pups were obtained for brain dissections but these animals did not have EEG recordings. For molecular analysis, mRNA expression levels were assessed in the MCx, SCx and thalamus using qPCR for the TARPs, (stargazin, γ3, γ 4, γ5, γ7, γ8) and AMPARs subunits GluA1 to GluA4. Results: There was no significant difference in stargazin mRNA expression in the SCx, MCx and thalamus in 7 day old GAERS that are not having spontaneous absence seizures compared to NEC rats. However, in 7 week old GAERS as seizures begin to manifest, stargazin mRNA is significantly increased in the SCx, MCx and thalamus ($p<0.05$ for the 3 regions). In adult epileptic GAERS the changes are more pronounced. In MCx, stargazin ($p<0.05$), γ3 ($p<0.01$), γ5 ($p<0.001$), and GluA4 ($p<0.05$) are increased, in SCx stargazin ($p<0.05$), γ3 ($p<0.01$), γ5 ($p<0.01$), γ8 ($p<0.001$) and GluA4 ($p<0.01$) are increased. In thalamus γ4 ($p<0.05$) and γ5 ($p<0.0001$) are increased. Conclusion: These results indicate that changes in TARP expression occur as seizures begin to manifest given that GAERS do not begin to express seizures until around 7 weeks of age, these findings establish a temporal association between increased TARPs expression and seizures.

Disclosures: P.M. Casillas-Espinosa: None. **R. Barmanray:** None. **T.J. O'Brien:** None. **K.L. Powell:** None.

Poster

247. **Epilepsy: Glutamatergic Transmission**

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Topic: C.08. Epilepsy

Support: 5 K08 NS58674

1 R01 NS070824

Title: Mechanisms underlying comorbid depression in temporal lobe epilepsy (tle)

Authors: *R. DHAHER, H. WANG, S. GRUENBAUM, C. ONG, H. ZAVERI, T. EID;
Lab. Med., Yale Univ., New Haven, CT

Abstract:
Depression represents one of the most common and serious comorbidities in patients with epilepsy. Glutamine synthetase (GS), an astrocytic enzyme, critical for metabolism of glutamine, glutamate, and GABA, is reduced in the amygdala in patients with epilepsy and also patients with depression (Kalkman, 2011). Here, we sought to develop a novel model of comorbid depression in TLE by microinfusion of the GS inhibitor methionine sulfoximine (MSO) unilaterally into the central nucleus of the amygdala (CeA) in rats. Male Sprague Dawley rats (380-420 g) were implanted with an osmotic pump injecting either MSO (n=7) or PBS (n=7) into the right CeA at a rate of 0.25 µl/hr. Intracranial EEG activity was monitored from the neocortex for a period of 21 days after the onset of MSO infusion. EEG analysis was correlated with video recordings to determine seizure severity according to the Racine scale. Sucrose preference testing to evaluate depressive-like behavior (anhedonia) occurred following EEG recording. Preference ratio was defined as (sucrose (mL))/ (H_2O (mL)). Recurrent seizures occurred over the three weeks of EEG testing. During the first three days following MSO surgery, 80% of seizures were non-convulsive, while during weeks 2 and 3, roughly 80% of seizures were convulsive (Racine stage 4-5). PBS-infused control rats showed a significantly higher preference ratio (7.93 ± 0.71) than MSO treated rats (2.40 ± 1.62) $p<0.05$ Sucrose consumption was also significantly higher in PBS treated rats (0.19 ± 0.05 g/kg) vs MSO-treated rats (0.08 ± 0.01) ($p<0.05$). H_2O consumption (mL) did not differ between PBS and MSO groups (2.47 ± 0.67 and 2.23 ± 0.87 respectively).This study suggests that perturbations in the glutamine-glutamate-GABA metabolism in the CeA are implicated in the causation of comorbid depression in TLE. We propose that the MSO CeA model can be used for mechanistic studies of comorbid depression in TLE and for testing of novel therapies of this condition.

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Poster

247. **Epilepsy: Glutamatergic Transmission**

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Cure Alzheimer's Fund

Tufts CNR P30NS047243

Title: Traumatic brain injury alters cortical glutamate network function by compromising GABAergic inhibition

Authors: *D. CANTU, K. WALKER, L. ANDRESEN, A. TAYLOR, D. HAMPTON, G. TESCO, C. DULLA;
Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Traumatic brain injury (TBI) is a major risk factor for developing pharmaco-resistant epilepsy. Although disruptions in cortical and hippocampal circuitry are associated with TBI, the precise mechanisms by which brain injury leads to epileptic network activity is unknown. In this study, we investigated how controlled cortical impact (CCI), a model of TBI, affects the spatial and temporal parameters of cortical glutamate network activity. Using FRET-based glutamate biosensors, we optically mapped cortical glutamate signaling in real-time while simultaneously recording cortical field potentials. 10 week old, male C57BL/6 mice underwent sham or CCI injury, and acute cortical brain slices were obtained 2-4 weeks post-injury; a time after injury but prior to the onset of spontaneous seizures. Slices from CCI injured cortex were loaded with glutamate biosensor immediately prior to imaging and recording. Results were compared to slices from sham injured mice. Electrical stimulation evoked polyphasic, epileptiform field potentials and disrupted input-output relationships in CCI injured cortex. High-speed glutamate biosensor imaging showed that glutamate signaling evoked by electrical stimulation was significantly increased in the injured cortex. Elevated glutamate responses were consistent with epileptiform activity, were highest in the area directly adjacent to the injury, and spread via deep cortical layers. Cortical glutamate release from CCI injured cortex was sequentially activated with the same temporal parameters as disinhibited control slices. Areas of cortical glutamate signaling were consistent with loss of GABAergic interneurons as measured by parvalbumin and somatostatin immunohistochemistry. Protein expression of the astrocytic glutamate transporters GLT-1 and GLAST showed no differences between sham and CCI injured slices. Our results suggest that loss of GABAergic inhibition in specific cortical sub-networks may facilitate the spread of epileptiform activity following TBI. Overall, our studies suggest that loss of inhibition after TBI leads to cortical network hyperexcitability which can ultimately result in posttraumatic epilepsy.

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Poster

247. **Epilepsy: Glutamatergic Transmission**

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Topic: C.08. Epilepsy

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CTSA Grant UL1 RR024139

Title: Characterization of brain structural changes in the methionine sulfoximine rat model of mesial temporal lobe epilepsy

Authors: *H. WANG[1], Y. HUANG[2], R. DHAHER[1], D. COMAN[2], H. ZAVERI[3], F. HYDER[2,4], T. EID[1];
[1]Lab. Med., [2]Diagnos. Radiology, [3]Neurol., [4]Biomed. Engin., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Patients with mesial temporal lobe epilepsy (MTLE) have often suffered from an insult to the brain several years before spontaneous recurrent (epileptic) seizures commence. This is thought to trigger a sequence of structural, molecular, and metabolic alterations that ultimately cause refractory epilepsy. The objective of the present study was to use brain diffusion tensor imaging (DTI) combined with neuropathological assessments to better define the structural alterations that underlie the development of MTLE.
Male Sprague Dawley rats were implanted with a 28-day osmotic pump that infused either the glutamine synthetase inhibitor methionine sulfoximine (MSO; n=7) or phosphate buffered saline (PBS; n=4) unilaterally into the deep entorhinal cortex at a flow rate of 0.25 uL/hr. This approach has been shown to result in epileptic seizures and neuropathological changes that resemble human MTLE. Video-intracranial electroencephalography (EEG) recordings performed in the third week following placement of the pump were used to monitor for seizures. Severity of seizures was characterized using a modified Racine Scale. Rats were perfusion fixed with 4% formaldehyde 6-8 weeks post-surgery and brains were scanned using a 9.4T horizontal bore magnet. Fractional anisotropy (FA) differences were subtracted from each group's average FA and then mapped onto an anatomical average of all brains. Brains were embedded in paraffin

after imaging, serially sectioned and processed for immunohistochemistry using antibodies against a variety of neuronal and glial components.
All of the MSO-infused rats and none of the PBS-infused rats developed recurrent seizures, which were predominantly of Racine stage 4-5. The MSO-infused rats showed extensive and *bilateral* changes by DTI in gray and white matter areas, as evidenced by *increased* FA in the thalamic nuclei and corpus callosum compared to PBS controls. Increases in FA were observed *unilaterally* in MSO vs. PBS treated rats. These increases were seen *ipsilateral* to the infusion site in the entorhinal cortex and CA3 field and *contralateral* to the infusion site in the amygdala. Since conventional MRI showed minimal lesions ipsilateral to the MSO infusion, most of the DTI-based changes are likely due to the effects of MSO. Correlations with immunohistochemistry are ongoing.
The MSO-model of MTLE exhibits significant bilateral alterations throughout the brain. The cellular and molecular correlates of these alterations are currently being investigated by immunohistochemistry. These findings are expected to provide new insights into the structural and molecular processes of epileptogenesis in MTLE.

Disclosures: H. Wang: None. **Y. Huang:** None. **R. Dhaher:** None. **D. Coman:** None. **H. Zaveri:** None. **F. Hyder:** None. **T. Eid:** None.

Poster

247. **Epilepsy: Glutamatergic Transmission**

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Topic: C.08. Epilepsy

Support: National Institutes of Mental Health (MH082881)

NDEPSCoR-DDA

Title: Corticotropin-releasing factor facilitates epileptiform activity via CRF2 receptor-mediated signaling mechanisms in the rat entorhinal cortex

Authors: *L. KURADA, N. I. CILZ, S. LEI;
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Abstract: Corticotropin-releasing factor (CRF) is a stress-released epileptogenic neuropeptide ubiquitously distributed in the brain, including the entorhinal cortex (EC). The EC is involved in the initiation, maintenance and spread of seizures. The role and relevance of CRF and its receptors in the EC is not known. We found that the EC expressed high levels of CRF and CRF2

receptors and bath application of CRF increased the frequency of picrotoxin-induced epileptiform activity recorded from layer III of the EC in slices, via CRF2 receptors and cyclic AMP, with a partial PKA involvement. Application of ZD 7288, a blocker of the hyperpolarization-activated channels (H-channels), significantly reduced the frequency of epileptiform activity but increased the numbers of the synchronizing events within single epileptiform activity and the duration of individual epileptiform activity. In the presence of ZD 7288, CRF failed to increase the frequency of epileptiform activity but still augmented the numbers of the synchronizing events in an epileptiform activity and the duration of epileptiform activity suggesting that part of the effects of CRF on epileptiform activity is mediated via H-channels. Furthermore, CRF increased H-channel currents recorded from layer II stellate neurons via activation of CRF2 receptors. Cyclic AMP not protein kinase A was responsible for CRF-mediated facilitation of H-channel currents.CRF caused membrane depolarization and increased action potential frequency of the principal neurons present in layer II and III of the EC, resulting in epileptiform activity.

Disclosures: L. Kurada: None. **N.I. Cilz:** None. **S. Lei:** None.

Poster

247. **Epilepsy: Glutamatergic Transmission**

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Topic: C.08. Epilepsy

Support: NIH Grant NS040272-11

Title: Assessing the presynaptic deficit in LIS1 mice

Authors: *W. B. POTTER, S. C. BARABAN;
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Abstract: Malformations of cortical development are associated with cognitive deficits and medically intractable epilepsy. Mutations in the gene encoding LIS1 cause Type 1 lissencephaly; a neuronal migration disorder that produces malformed cortical and subcortical structures resulting in hyperexcitable and epileptic activity. LIS1 heterozygous mutant mice recapitulate many structural and cellular hallmarks of human lissencephaly, namely: electrographic seizures, a disordered hippocampus, abnormal neurogenesis and migration, and enhanced excitatory drive to principal hippocampal neurons. Interestingly, our lab recently found that conditional, postnatal knockout of LIS1 (LIS1-cko) also produces over-excitation in the hippocampus, suggesting that LIS1 deficiency is pro-epileptic in the absence of structural malformation. This project addresses

the causal role of presynaptic dysfunction in the development of hyperexcitability due to LIS1 deficiency. To this end we applied pharmacological modulators of presynaptic function and vesicle formation, and assessed the impact on excitatory transmission at CA3-CA1 hippocampal synapses. This was accompanied by biochemical analysis of presynaptic protein expression and signaling cascades. With the ultimate goal of finding new approaches towards treating malformation-associated epilepsy, this project seeks to determine whether the manipulation of presynaptic function can correct for key pathological phenotypes in a mouse model of malformation.

Disclosures: W.B. Potter: None. **S.C. Baraban:** None.

Poster

247. **Epilepsy: Glutamatergic Transmission**

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Topic: C.08. Epilepsy

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CIHR Grant MFE115462

Title: Early life seizures impaired homeostatic synaptic scaling in cortical neurons

Authors: *H. SUN[1,2], B. KOSARAS[2], F. E. JENSEN[1,2];
[1]Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA; [2]Boston Children's Hosp. and Harvard Med. school, Boston, MA

Abstract: Both Hebbian and homeostatic synaptic plasticity are most robust during neonatal period, in which the incidence of seizures is one of the highest across the lifespan. Neonatal seizures are often refractory to conventional antiepileptic drugs, and can result in later life epilepsy, cognitive deficits and autism. We have previously shown that early-life seizures (HS) result in acute and long-term enhancement of AMPAR function and impairment in LTP. In addition to Hebbian synaptic plasticity, homeostatic synaptic scaling calibrates neuronal excitability by adjusting synaptic strengths during prolonged changes in synaptic activity. We have recently shown that HS itself can evoke PLK2-mediated homeostatic modulation of seizure-induced enhancement of AMPAR function. Here we hypothesize that neonatal seizures

may alter homeostatic synaptic scaling in epileptic neurons in response to further chronic activity changes. Neonatal Hypoxic seizures were induced by graded global hypoxia at postnatal day 10. Ex vivo slices were maintained in vitro for 48-72h using an acute culture procedure. Whole-cell patch clamp recordings of AMPAR mEPSCs were made in cortical neurons from post-HS 48h rats and their littermate controls. Compared to acute slices, acute cultured slices did not show significant changes in intrinsic membrane properties and amplitude and frequency of AMPAR mEPSCs ($p>0.05$, n=8-9). We found that chronic activity blockade by 1μM TTX for 24-48h induced significant increases in amplitude and frequency of AMPAR mEPSCs in neurons from both HS rats and normoxic controls ($p<0.05$, n=6-8). However, the decrease in synaptic strength in response to elevated synaptic activity by application of 100μM Picrotoxin 24-48h as identified in controls ($p<0.05$, n=6-8) was occluded in neurons from HS rats ($p>0.05$, n=7-8). Overall, our data reveal an impaired homeostatic synaptic scaling down in neurons following early-life seizures, which may, at least in part, contribute to neonatal seizure-induced long-term neuronal hyperexcitability and epileptogenesis.

Disclosures: H. Sun: None. **B. Kosaras:** None. **F.E. Jensen:** None.

Poster

247. **Epilepsy: Glutamatergic Transmission**

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Support: Funded by GW Pharmaceuticals plc

Title: The anticonvulsant phytocannabinoid cannabidivarin (CBDV) has a disease-dependent effect on synaptic transmission

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Abstract: Cannabidivarin (CBDV) is a non-psychoactive phytocannabinoid and propyl analogue of cannabidiol (CBD). We previously demonstrated that CBDV reduces epileptiform activity in vitro and shows significant anticonvulsant actions in vivo (Hill et al., 2012 Br. J. Pharmacol. 167:1629-42). However, little is currently known about CBDV's anticonvulsant mechanism of action. Here, we investigate CBDV effects on synaptic transmission in hippocampal tissue from both control and epileptic rats.
Acute transverse, hippocampal slices (300 μm) were taken from 3 groups of male Wistar rats:

young controls (P24-28), rats with spontaneous recurrent seizures (induced by pilocarpine treatment and behaviourally confirmed to have epilepsy) and age-matched controls (~P50-60). Pairs of evoked local field potentials (LFPs; inter-stimulus interval 50 ms) were recorded from the stratum radiatum of the CA1 region using multi-electrode arrays (MEAs) and the ratio of paired LFP amplitudes (paired pulse ratio; PPR) was obtained. Independently, whole cell patch clamp of CA1 pyramidal cells was used to record miniature inhibitory postsynaptic currents (mIPSCs).

PPR in epileptic tissue was significantly greater than PPR in age-matched controls (1.65 ± 0.1 vs 1.36 ± 0.05; $p \leq 0.01$). CBDV (1-50 μM) significantly reduced PPR in both P24-28 and ~P50-60 control tissues (10-22%; $p < 0.05$), but had no effect on PPR in epileptic tissue. Miniature IPSC amplitude in P24-28 control slices was not significantly different when compared to epileptic slices (18.9 ± 5.1 pA vs 20.2 ± 6.7 pA). However, mIPSC frequency in epileptic tissue was significantly reduced when compared to P24-28 control tissue (0.4 ± 0.1 Hz vs 1.0 ± 0.2 Hz; $p \leq 0.05$). CBDV (10 μM) had no significant effect on mIPSC amplitude and frequency in both P24-28 control and epileptic slices.

These data demonstrate that epileptic and non-epileptic tissues have differences in synaptic transmission and that the effect of CBDV could be disease-dependent. In non-epileptic tissue, results were consistent with CBDV increasing the probability of presynaptic neurotransmitter release in hippocampus CA1. However, CBDV does not alter release probability in epileptic tissue or change action potential-independent GABAergic transmission. The interaction between disease-dependent changes in both intracellular calcium handling and responses to CBDV remain the focus of our future work.

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Poster

247. **Epilepsy: Glutamatergic Transmission**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 247.13/W15

Topic: C.08. Epilepsy

Support: Beca CONACyT

PAEP-UNAM

Title: Changes in the expression of MDR1 and MRP1 by probenecid administration to reduce NMDA receptor activity in kindled rat

Authors: *C. C. LIVERA[1], J. MARTÍNEZ-LAZCANO[2], E. GONZÁLEZ-GUEVARA[2], D. CAMPOS - ARROYO[4], V. CUSTODIO RAMÍREZ[2], M. HERNÁNDEZ - CERÓN[2], M. RUBIO[2], F. PÉREZ - SEVERIANO[3], C. PAZ[2];
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Abstract: The therapeutic strategies on the refractory epilepsy, points to the coadministration of multidrug resistant proteins (MDR1 and MRP) inhibitors -like probenecid-, with antiepileptic drugs. There is no data that correlates MDR1 and MRP, with the activity of NMDA receptor; the main excitatory receptor involved in the epileptic activity, and in the development of *kindling.* The kindling model involves the activation of NMDA receptor for the development of 5 class of seizures, ending up in general seizures (1-5 in Racine's scale). In this study, male Wistar rats were electrically stimulated for the development of kindling. Then different doses of probenecid were administrated (75, 100 y 300 mg/kg) for 5 days. After the treatment, the rats were sacrificed by decapitation and the piriform cortex and hippocampus were dissected, for the analysis of the expression of MRP1, MDR1 and NR2B subunit of NMDA receptor; as well as the activity of nitric oxide synthase (NOS), as an indirect marker of NMDA receptor activity. The results shows a decrease in the after discharge time, and a 75% reversion of the seizure class (5-3). The cDNA levels indicate a decrease in the ARNm of MDR1 gene, but not in MRP1. The Western Blot analysis shows a decrease in the MDR1, MRP1 and NR2B expression levels. The NOS activity was reduced. The differential effect of the different doses of probenecid might be explained by the reduction of the expression of MRP1, MDR1.

Disclosures: C.C. Livera: None. **J. Martínez-Lazcano:** None. **E. González-Guevara:** None. **D. Campos - Arroyo:** None. **V. Custodio Ramírez:** None. **M. Hernández - Cerón:** None. **M. Rubio:** None. **F. Pérez - Severiano:** None. **C. Paz:** None.

Poster

247. **Epilepsy: Glutamatergic Transmission**

Location: Halls B-H

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Topic: C.07. Developmental Disorders

Title: Hippocampal synaptic plasticity after development in a rat model of cryptogenic infantile spasms

Authors: M. TSUJI, Y. TAKAHASHI, A. M. WATABE, *F. KATO;
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Abstract: Infantile spasms (IS), a specific type of seizure in a catastrophic epilepsy syndrome of infancy and childhood, is strongly associated with cognitive impairment and psychosocial morbidity in adolescence and adulthood despite seizure remission. The etiology as well as the mechanism underlying such remote influence of cryptogenic IS to the higher brain function remains unidentified. To address this issue, we made an animal model of cryptogenic IS according to a method recently developed by Velisek et al (2010) and analyzed the short- and long-term synaptic plasticity in the hippocampus of young adult rats. Pregnant Sprague-Dawley rats were treated with betamethasone (0.4 mg/kg, i.p.) or saline (saline group) and the offsprings were injected with NMDA (15 mg/kg, i.p.) three times on postnatal days 12 to 15 (NMDA group). In accordance with previous reports, these injections resulted in typical flexion spasms with irregular EEG spike bursts lasting 30-60 minutes. A subset of animals was treated with ACTH before NMDA injections (2-3 times for 3 days; ACTH group). After weaning, the rats were raised with normal chow and water until postnatal week 6-8, at which hippocampal slices were prepared from these groups of animals. The paired-pulse ratio, as estimated from the slope of field EPSP in CA1 evoked by paired stimulation of the Schaffer collateral (interval, 50 ms), was significantly smaller in NMDA group than in ACTH group in female rats. Post-tetanus potentiation observed after 100-Hz stimulation for 1 s delivered twice separated by 10 s was weakest in NMDA groups. The differences in release properties between groups were not confirmed in the male rats. The NMDA group also showed the smallest long-term potentiation as measured at 35-45 min after the tetanus. These results indicate that IS-like aberrant activity in the early stage of life would result in remote effects including altered short-term plasticity in the hippocampus, appearing later in life.

Disclosures: M. Tsuji: None. **F. Kato:** None. **Y. Takahashi:** None. **A.M. Watabe:** None.

Poster

248. **Epileptogenesis**

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Topic: C.08. Epilepsy

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Title: Alterations of TLR4 expression following brain injuries

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Abstract: Brain injury is the major cause of acquired epilepsy and diabetic patients have higher incidence of seizures. Accumulating evidence suggests that toll-like receptor 4 (TLR4) plays an important role in pathogenesis of epilepsy following brain injury. We investigated the TLR4 expression after ischemia under hyperglycemia and following traumatic brain injury.
Traumatic brain injury (TBI) was induced in Sprague Daelet rats (~300g) by controlled cortical impact (CCI) method with a pneumatic piston containing a 3.0 mm diameter tip at a rate of 2.7 m/s and 1.0 mm of compression. Global ischemia was produced in adult Wistar rats using four-vessel occlusion method for 15 min. Hyperglycemia (> 200 mg/dL) was induced by intraperitoneal injection of glucose (3.0 g/kg) 15 min before ischemia. The animals were sacrificed at different time points after TBI or ischemia. The neuronal death in the hippocampus was determined by hematoxylin-eosin staining. The expression levels of TLR4 and HMGB1 were analyzed by Western blotting and immunohistochemistry. Inflammation induced by Lipopolysaccharide (LPS, i.p.) was used as positive controls.
In naïve animals, TLR4 was weakly expressed in hippocampus and HMGB1 was localized in the nucleus of neurons throughout the hippocampus. In ischemia studies, the intensity of TLR4 immunostaining was significantly increased in hippocampal interneurons in hyperglycemic group after ischemia as compared with normoglycemic and control group. Meanwhile, a dramatic decrease of HMGB1 expression was observed in the hippocampus after hyperglycemic ischemia. No difference in the expression of TLR4 and HMGB1 was observed between

hyperglycemic group and LPS group. The immunoreactivity of c-fos was also increased in hippocampal neurons in hyperglycemic animals after ischemia, suggesting the increase of neuronal activity. Most rats in the hyperglycemic group developed tonic-clonic seizures after ischemia. The animals with seizures died of status epilepticus within 2 h after the onset of seizure. In TBI studies, the cortical impacts produced a lesion in the ipsilateral cortex. However, no obvious neuronal death was found in the ipsilateral hippocampus up to 3 days after TBI. Immunohistochemical staining and Western blotting indicated a significant increase of TLR4 expression in the ipsilateral hippocampus, but not in the contralateral hippocampus. The increase of TLR4 expression reached the peak at 6 hrs after TBI.
These results suggested that up-regulation of TLR4 might contribute to the pathogenesis of seizures/epilepsy following brain injuries.

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Poster

248. **Epileptogenesis**

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Topic: C.08. Epilepsy

Support: CIHR

University of Calgary

Hotchkiss Brain Institute

Sembo Family

Title: Seizure-induced severe ischemic/hypoxic episodes (SISIHE) explains Todd's paresis

Authors: *J. S. FARRELL[1,2], J. F. DUNN[2], G. C. TESKEY[2];
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Abstract: Brief seizures cause severe local brain tissue hypoxia (pO2 <10mmHg) immediately after seizures that is linearly related to the duration of the seizure and that often lasts for over an hour in the hippocampus and neocortex. We also have observed that the resulting hypoxia is accompanied by reduced blood perfusion and, therefore, termed this novel phenomenon Seizure-Induced Severe Ischemic/Hypoxic Episode (SISIHE). In some people with epilepsy the postictal period is often marked by specific behavioural impairments. Todd's Paresis, for example, is

defined by motor weakness or paralysis after a seizure and has previously been attributed to neuronal exhaustion or motor neuron inactivation. Given that SISIHE and postictal deficits occur on similar time-scales, we hypothesized that the resulting hypoxia after seizures is responsible for the ensuing behavioural deficits. We tested this hypothesis by preventing vascular smooth muscle contraction with nifedipine and thus, dissociated the severe hypoxia from a neocortical-kindled seizure. We assessed grip strength on the hanging bar test before the seizure, during the post seizure hypoxic period (20 and 40 minutes) and following recovery of local tissue oxygen (80 minutes). Vehicle injected rats held onto the bar for a significantly shorter time during the hypoxic period at 40 minutes but recovered after return to normoxia (80 minutes). Nifedipine pretreatment was able to prevent both the hypoxia and grip-strength deficit without affecting seizure duration. This experiment provides evidence that postictal behavioural deficits are a result of prolonged insufficient oxygen and can be prevented with pharmacological agents that prevent reduced blood perfusion. Clinicians should consider treating Todd's paresis and other postictal behavioural impairments to prevent the consequences of hypoxic episodes.

Disclosures: J.S. Farrell: None. **J.F. Dunn:** None. **G.C. Teskey:** None.

Poster

248. **Epileptogenesis**

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Program#/Poster#: 248.03/X1

Topic: C.08. Epilepsy

Support: NSF Grant IOS1021860

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Title: Role of RNA-Polymerase-1 in epileptogenesis

Authors: *M. HETMAN[1], A. VASHISHTA[1], R. PARLATO[2];
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Abstract: Hyperactivation of the mTOR pathway and the following deregulation of brain cell growth including neuronal hypertrophy have been implicated in epileptogenesis. RNA-Polymerase-1 (Pol1)-mediated transcription of the nucleolar rRNA genes is the critical regulatory step of ribosomal biogenesis. The Pol1/ribosomal biogenesis pathway is stimulated by the ERK1/2 MAP kinase and the mTOR to promote proliferative cell growth. To investigate the role of ribosomal biogenesis in development of epilepsy, effects of increased neuronal activity on nucleolar transcription were determined in primary cultures of rat forebrain neurons and in the

hippocampus of mice that were subjected to seizures. In cultured cortical or hippocampal neurons gabazine stimulated Pol1-mediated transcription in an NMDA receptor-dependent manner. In whole mice, a transient seizure episode induced by the pro-convulsant pentylenetetrazole (PTZ) or status epilepticus in response to kaininc acid stimulated Pol1 and increased expression of the Pol1 co-activator Ubf. Moreover, mice that had a tamoxifen-induced knockout of the essential Pol1 co-activator Tif1a that was limited to excitatory forebrain neurons were resistant to PTZ kindling. These findings suggest that nucleolar transcription is regulated by seizures to promote development of epilepsy. Hence, ribosomal biogenesis may be a novel target for anti-epileptogenic interventions.

Disclosures: M. Hetman: None. **A. Vashishta:** None. **R. Parlato:** None.

Poster

248. **Epileptogenesis**

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Topic: C.08. Epilepsy

Support: R37 NS35439

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Title: Dysregulated NRSF expression after epilepsy-inducing insults is caused by a loss of microRNA-124

Authors: *G. P. BRENNAN[1], S. MCCLELLAND[1], S. IYER[1], T. Z. BARAM[1,2];
[1]Pediatrics, [2]Anat. & Neurobio., Univ. of California Irvine, Irvine, CA

Abstract: Rationale: Neuron restrictive Silencing factor (NRSF/REST) is a transcriptional repressor of neuronal genes in non-neuronal tissue, found at very low levels in neuronal tissue. Our previous studies found upregulation of NRSF within hippocampus during epileptogenesis that follows kainic-acid (KA)-induced status epilepticus (SE), resulting in the repression of a number of key neuronal genes. Interference with NRSF function using an oligodeoxynucleotide decoy strategy resulted in rescue of the repressed genes and, importantly, in attenuating epileptogenesis (McClelland et al, 2011). These data provided strong support for a role of NRSF upregulation in epileptogenesis, raising the question: How do epilepsy-provoking insults upregulate NRSF expression and function?
Methods: Status epilepticus was induced in P10 rats by systemic KA administration. Because NRSF might be regulated by microRNAs (Packer et al., 2008), and because reduced miR124 was

found in epileptic brain (Kan et al., 2012), we examined levels of NRSF, REST4 and miR124 levels at specific time points using qRT-PCR. To study directly whether altered levels of miR-124 regulate NRSF expression, organotypic hippocampal slice cultures were treated with KA to produce long (3h) seizure-like events. Hippocampi were then exposed to either miR-124 mimics or scrambled mimic controls for 48h and expression levels of NRSF, REST4 and a target gene of NRSF, HCN1, were determined using qRT-PCR.
Results: NRSF mRNA levels increased enduringly following SE, confirming our previous reports. Levels of miR-124 decreased as early as 60 min following KA administration, prior to reduction of NRSF levels,and corelated inversely with those of NRSF throughout the experiment. Whereas total NRSF levels increased, the REST4/NRSF ratio was reduced, suggesting lower production of alternatively spliced isoforms of NRSF. Repression of HCN1, a target gene of NRSF, was augmented after the KA-seizures, suggesting augmented NRSF function resulting from increased repressor levels. A mimic of miR-124, given after the long seizure, attenuated increases in NRSF levels and prevented repression of the NRSF target gene HCN1.
Conclusions: Together, these data support the scenario that seizures repress miR-124 levels, resulting in enhanced NRSF levels and function. Because augmented NRSF repression of key neuronal genes promotes epilepsy, miR-124 based therapeutics may provide a selective strategy option in the prevention of epileptogenesis.

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Poster

248. **Epileptogenesis**

Location: Halls B-H

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Topic: C.08. Epilepsy

Support: Mid-Career Researcher Program through the National Research Foundation of Korea (NRF) grant funded by the MEST (2011-0028319)

Title: Aspirin prevents the development of hippocampal mossy fiber sprouting through the attenuation of mTOR signaling in a mouse model of temporal lobe epilepsy

Authors: *K. JEONG[1,2], J. KIM[1,2], M.-Y. LEE[3,2], S. KIM*[1,2];
[1]Departments of Pharmacol., Col. of Medicine, The Catholic Univ. of Korea, Seoul, Korea, Republic of; [2]Catholic Neurosci. Inst., [3]Departments of Anat., The Catholic Univ. of Korea, Seoul, Korea, Republic of

Abstract: Aspirin, a widely used non-steroidal anti-inflammatory drug (NSAID), reportedly suppresses reorganization of mossy fibers after epileptic insults. In addition, a recent study suggested that mammalian target of rapamycin (mTOR) signaling might be involved in the mossy fiber sprouting related to status epilepticus (SE). Thus, the present study was performed to investigate whether aspirin affects the mTOR signaling pathway to attenuate mossy fiber sprouting after pilocarpine-induced SE. Aspirin was daily administrated (15 mg/kg or 150 mg/kg, i.p.) for 10 days starting from 3 days before SE, continuing until 6 days after SE. In order to induce SE, C57BL/6 mice (aged 8 weeks) were administrated with pilocarpine hydrochloride (280 mg/kg, i.p.) at 30 min after pretreatment with atropine methyl nitrate (2 mg/kg, i.p.) and terbutaline hemisulfate (2 mg/kg, i.p.). Mossy fiber sprouting was measured in the hippocampus using Timm staining. Phosphorylated S6 ribosomal protein (pS6) was used as a biomarker of mTOR activity. Seven days after SE, mossy fiber sprouting was significantly decreased in the high dose (150 mg/kg) aspirin-treated group as compared to the vehicle-treated control group. In addition, treatment with high dose aspirin significantly reduced the number of pS6-positive cells which was increased in the dentate granule cell layer of the hippocampus of vehicle-treated mice at 7 days after SE. These findings suggest that aspirin could attenuate mossy fiber sprouting, probably through inhibition of mTOR signaling, after pilocarpine-induced epilspy.

Disclosures: K. Jeong: 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.; Mid-Career Researcher Program through the National Research Foundation of Korea (NRF) grant funded by the MEST (2001-0028319). **J. Kim:** None. **M. Lee:** None. **S. Kim*:** None.

Poster

248. **Epileptogenesis**

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Program#/Poster#: 248.06/X4

Topic: C.08. Epilepsy

Support: KAKENHI 22500316

Title: Chromatin remodeling in neurons in the caudate-putamen after excessive neuronal excitation

Authors: T. MORI, *T. WAKABAYASHI, Y. HIRAHARA, Y. TAKAMORI, T. KOIKE, H. YAMADA;
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Abstract:

Status epilepticus (SE) induces pathological and morphological changes in the brain. Recently, it has become clear that excessive neuronal excitation, stress and drug abuse induce chromatin remodeling in neurons, thereby altering gene expression. Chromatin remodeling is a key mechanism of epigenetic gene regulation. Histone H3 phosphorylation is frequently used as a marker of chromatin remodeling and is closely related to the upregulation of mRNA transcription. In the present study, we analyzed H3 phosphorylation levels *in vivo* using immunohistochemistry in the brains of mice with pilocarpine-induced SE. A substantial increase in H3 phosphorylation was detected in neurons in specific brain structures. Increased H3 phosphorylation was dependent on neuronal excitation. In particular, a robust upregulation of H3 phosphorylation was detected in the caudate putamen, and there was a gradient of phosphorylated $H3^+$ ($PH3^+$)
neurons along the medio-lateral axis. After unilateral ablation of dopaminergic neurons in the substantia nigra by injection of 6-hydroxydopamine, the distribution of $PH3^+$ neurons changed in the caudate putamen. Moreover, our histological analysis suggested that, in addition to the well-known MSK1 (mitogen and stress-activated kinase)/H3 phosphorylation/c-fos pathway, other signaling pathways were also activated. Together, our findings suggest that a number of genes involved in the pathology of epilepsy are upregulated in $PH3^+$ brain regions, and that H3 phosphorylation is a suitable indicator of strong neuronal excitation.

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Poster

248. **Epileptogenesis**

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Topic: C.08. Epilepsy

Title: Transcriptome profiling of hippocampal CA3 reveals spatially distinct patterns of gene expression after early-life seizure-induced preconditioning

Authors: *S. HU, A. M. SLOMKO, Z. NASEER, S. S. ALI, Y. GLICKSMAN, L. K. FRIEDMAN;
Cell Biol. and Anat., New York Med. Col., Valhalla, NY

Abstract: The immature brain is relatively resistant to seizure-induced injury. Moreover, multiple early-life seizures result in spatial preconditioning. For example, injury to the CA1 but not CA3 hippocampal subregion of juvenile animals (P20) is attenuated following a single injection of kainic acid (KA) ($1\times KA_{p20}$) if animals have a history of two sustained neonatal seizures on P6 and P9 ($3\times KA_{p20}$). The underlying mechanisms of age-dependent, spatially distinct neuroprotection and the responsible major signaling cascades remain unknown. In order to identify gene candidates involved in the age-dependent and spatially protective effects, we previously profiled transcriptomes of the isolated CA1 subregion after $1\times KA_{p20}$ and $3\times KA_{p20}$. The present study isolated transcriptomes of the CA3 subregion under three conditions [after $1\times KA_{p20}$, $3\times KA_{p20}$, and 3×KA on P6, P9 and P13 ($3\times KA_{p13}$)]. Under all three conditions, 11346 genes (Agilent platform) were regulated: $1\times KA_{p20}$ (22.1%) vs. $3\times KA_{p20}$ (50.2%), vs. $3\times KA_{p13}$ (28.7%). A large number of genes were uniquely regulated, of which only 830 were commonly upregulated and 290 commonly downregulated when comparing the P20 group. More genes were upregulated in the CA3 subregion after $3\times KA_{p20}$ compared to $1\times KA_{p20}$ and $3\times KA_{p13}$. Moreover, many more genes were downregulated in the CA3 compared to the CA1 after $3\times KA_{p20}$ (Duke Platform) (1393 vs. 41). Although many autophagy genes were triggered by single or multiple seizures within the CA3, many protective genes were also differentially upregulated, particularly after 3×KA in both age groups, but with different expression profiles. These included, but were not limited to, calcium modulated proteins (e.g. annexins, calmodulin), apoptosis inhibitors (e.g. anti-apoptotic Bcl2 members), adaptor-related protein complexes, ADAM metalloproteinases, adaptor ATG autophagy genes, caspase cascade activators, ATP-mediated gliotransmitters, GTP binding proteins, cyclins, F-box proteins, growth factors, interleukins, heat shock proteins, and certain ionotropic and metabotropic glutamatergic and GABAergic neurotransmitter receptors and synthesizing enzymes. Examples of genes that were differentially downregulated between the groups include ankyrin-repeat proteins, adenosine receptors, ATP synthases, caspase recruitment domains, calcium channels, certain heat shock proteins, NFKB activating proteins, synaptosomal associated proteins, potassium voltage gated channels, and zinc finger domains. Age and regional differences in gene expression are likely responsible for early-life resistance to injury as well as the early-life seizure-induced neuroprotective effects.

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Poster

248. **Epileptogenesis**

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Program#/Poster#: 248.08/X6

Topic: C.08. Epilepsy

Support: FAPESP grant #2011/18294-5

Title: May neonatal seizures plant the roots for epileptogenesis?

Authors: *E. Y. SONODA[1], M. J. G. MARQUES[1], E. A. CAVALHEIRO[1], R. M. CYSNEIROS[2], F. A. SCORZA[1];
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Abstract: Seizures in newborns (NBs) remain the most frequent neurological problem in the nursery. Although seizures per se do not cause irreversible damage in the immature (developing) brain, NB seizures may immediately alter some electrical parameters and behavioral patterns. However, it remains unclear whether or not NB who had seizures will be more prone to develop epilepsy later in life. The effects caused by seizures in this population can be either transient or persistent, and the long-term effects may occur even with no histological evidence of neuronal injury. In order to address the unresolved question of whether seizures in NBs can plant the roots for epileptogenesis, the objective of this research was to analyze whether a status epilepticus (SE) in the NB facilitates the amygdala kindling process in the adult period of life. Firstly, nine days postnatal Wistar rats were divided into two groups: one was injected with pilocarpine solution (380mg/kg) and the other was injected with saline solution (control group). The animals injected with pilocarpine had a single SE and did not present spontaneous recurrent seizures thereafter, while control animals presented no SE and no spontaneous seizures. At the sixtieth day postnatal, all animals were submitted to the electrical amygdala kindling process. The number of stimulus required to complete the kindling model of epilepsy (3 consecutive seizures at stage 5, as described by Racine 1972), the time of afterdischarges (AD) and afterdischarge threshold (ADT) necessary for seizure initiation were analyzed. The results showed that the animals which presented SE needed a smaller number of stimuli to complete the kindling process than the control animals. Although the number of stimuli was different, the AD and ADT did not differ between groups. Additionally, animals submitted to the SE in the neonatal period firstly presented seizures classified as stage 2 of the kindling model, thereby bypassing stage 1 seizures. In conclusion, unlike in the adult brain, SE in the NB may lead to neuroplastic effects that protect the brain from recurrent seizures, as the animals injected with pilocarpine solution did not present spontaneous recurrent seizures and the AD and ADT was not different between experimental and control animals. On the other hand, the kindling process was faster in the experimental group than in the control animals, which suggests that the SE in the NB may lead to some alterations in the brain, probably as a consequence of disrupted cortical synapses, myelin formation in the cerebral hemispheres, and formation of the long tracts.

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Poster

248. **Epileptogenesis**

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Fédération de la Recherche sur le Cerveau (FRC)

Fondation Jérôme Lejeune

Title: Exposure to caffeine during pregnancy and lactation increases network excitability and seizure susceptibility in rodent offspring

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Abstract: Purpose: Consumption of psychoactive drugs during pregnancy interferes with brain development leading to deleterious long-term psychiatric, neurological and cognitive impairments in offspring. Caffeine, the most widely consumed psychoactive substance, is a naturally-occurring antagonist of adenosine receptors, which regulate numerous intracellular processes. Its effect on brain development is controversial. We tested whether maternal caffeine exposure in mice dams affected the physiology of their offspring.
Method: Dams were exposed to 0.3 g/L caffeine in their drinking water during pregnancy and lactation. In vivo recordings were performed with silicon probes in caffeine-exposed and control pups during the first postnatal week in the hippocampus and visual cortex V1. Seizure susceptibility was assessed with PTZ.
Results: Caffeine exposed pups displayed network hyperexcitability in vivo and reduced threshold to PTZ-induced seizures.
Conclusion: Caffeine exposure during pregnancy and lactation leaves a mark in neuronal circuits rendering them hyperexcitable. Maternal ingestion of caffeine during pregnancy and lactation may constitute a risk factor for the development of seizures in human neonates.

Disclosures: A.W. Fazeli: None. **A. Ghestem:** None. **S. Marguet:** None. **R. Cunha:** None. **M. Esclapez:** None. **D. Isbrandt:** None. **C. Bernard:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Academy of Finland

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Title: Post-traumatic epileptogenesis in APP/PS1 mouse model of Alzheimer`s disease

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Abstract: Amyloidogenesis is a common pathology in traumatic brain injury (TBI) and Alzheimer`s disease (AD), both of which associate with an elevated risk of epilepsy. Moreover, TBI is a risk factor for AD, facilitating amyloidogenesis. To elaborate the role of increased amyloid-β load on post-TBI epileptogenesis we investigated whether traumatic brain injury (TBI) facilitates epileptogenesis in an AD mouse model, and whether that associates with acquired channelopathy.
TBI was triggered using control cortical impact (CCI) in 13-15 wk old male APP/PS1 mice (n=14) and their wild type (Wt) littermates (n=17). Mice were assessed with composite Neuroscore for motor recovery at 2 d, 7 d, and 14 d post-TBI. Spatial memory was evaluated using Morris water-maze (MWM) at 14 d post-TBI. Then, mice were followed-up for 2 wk (24h/7d) with continuous video-EEG monitoring starting at 6 wk and 14 wk post-TBI to assess occurrence of spontaneous seizures and epileptiform discharges (EDs). At the end, mice were sacrificed and gene expression profiling of perilesional cortex, ipsilateral thalamus and ipsilateral hippocampus was performed using Affymetrix microarray system (Affymetrix Mouse Gene 2.1 ST Array).
APP/PS1 injured mice showed motor deficits at 2 d, 7 d and 14 d post-TBI as compared to

APP/PS1 controls ($p<0.01$). APP/PS1 injured mice were more impaired than Wt injured mice at 14 d post-TBI ($p<0.01$). Latency to find the platform in MWM was longer in APP/PS1 injured mice than in the Wt injured group ($p<0.05$). In the probe trial, APP/PS1 injured mice spent less time in the target quadrant than APP/PS1 controls ($p<0.05$). Video-EEG monitoring performed at 6 wk post-TBI revealed spontaneous seizures in 86% of APP/PS1 injured animals and 36% of APP/PS1 controls ($p<0.05$). In Wt groups, 7% of Wt injured mice and none of Wt controls displayed spontaneous seizures (p0.05). Neither Wt injured mice nor Wt controls had spontaneous seizures. The number of EDs did not differ between the groups. Preliminary microarray data analysis revealed changes in transcriptome between the groups.
Enhanced amyloidogenesis results in more pronounced epileptogenesis and more severe motor and cognitive co-morbidities following TBI.

Disclosures: D. Miszczuk: None. **H. Tanila:** None. **K. Lukasiuk:** None. **A. Pitkanen:** None.

Poster

248. **Epileptogenesis**

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Program#/Poster#: 248.11/X9

Topic: C.08. Epilepsy

Support: NIH-NINDS 220804

NIH-NINDS 217807

Title: Long-term effects of the initial pattern of activity-dependent synaptic depression on seizure probability in a hippocampal network model

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Abstract: Using a large scale computational model of hippocampal area we modeled changes in network activity following trauma and during epileptogenesis. The model was comprised of a 105 x 105 array of integrate-and-fire neurons synaptically connected via glutamatergic connections, and a 18 x 18 array of interneurons with GABAA outputs. For each modeling session, inputs and outputs were stochastically generated using the same connectivity probabilities, so that each session was comprised of a different instantiation of the core network. Fractional cell loss due to trauma was equally and randomly distributed in both glutamatergic and GABAergic networks. After cell loss followed by recurrent axon sprouting networks developed bursts of synchronous activity. Axon sprouting continued until surviving cells to

reconstituted original number of synapses. As a result bursts increased in frequency and in fraction of participating neurons, sometimes generating sustained (tonic) ictal-like activity that slowly transitioned into intermittent (clonic) activity.
We then used this model to address two questions: 1) What factors other than the pattern of sprouting contribute to the transformation from periodic synchronous bursting to ictal-like activity? 2) What factors contribute to the transformation of ictal-like activity from the tonic to clonic phase? We analyzed spatial and temporal patterns of synchronous activity propagation, synaptic depression and recovery, and local and global imbalances between inhibition and excitation. Our findings include 1) recurrent sprouting is necessary but not sufficient for ictal-like activity. 2) In networks with identical connectivity, transition from bursting to ictal activity depended on the initial conditions, specifically the distribution of activity-dependent synaptic depression. 3) Transition from tonic to clonic activity and eventually back to periodic synchronous bursting was not caused by global activity-dependent synaptic depression, but depended instead on the anatomical distribution of synaptic depression and recovery. 4) Differences governing these transitions were relatively small and it was surprisingly difficult to correlate them with obvious changes in network activity.
These simulations provide insight into our difficulties understanding and controlling ictogenesis. Understanding ictogenesis requires not only knowledge of connectivity but also subtle, activity-dependent inhomogeneities permissive for seizure onset. Similarly, control of seizure onset would require precise knowledge of extensive areas of network with high temporal resolution that is currently not technically feasible.

Disclosures: W.B. Swiercz: None. **K.J. Staley:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

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Topic: C.08. Epilepsy

Support: 1 R01 NS059740

Title: Homeostatic synaptic scaling mediates distinct types of paroxysmal activity following brain trauma

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Abstract: Epilepsy is commonly observed following brain trauma, however mechanisms that cause epileptic seizures are still not fully understood. Experimental and modeling studies suggested that homeostatic scaling of synaptic efficacy due to deafferentation resulting from head trauma could lead to epilepsy. Epileptic seizures involve large changes in ion concentrations, however, it remains unknown how homeostatic changes interact with ion concentration dynamics during interictal and ictal periods. In this study, we examined the effect of homeostatic synaptic scaling in a biophysically realistic cortical network model that included the dynamics of extra- and intracellular ion (Na+, K+ and Cl-) concentrations. In the model, trauma was induced by partial deafferentation of neurons and excitatory connections between neurons were then scaled by homeostatic processes to maintain a firing rate of about 5 Hz. We observed two distinct types of abnormal activity following deafferentation and synaptic scaling. Increases in synaptic strength led to intermittent periods of high frequency activity accompanied by a small increase in extracellular K+ concentration (from 3 to 4.5 mM) and followed by periods of low activity; this network state did not transition to full-scale seizures. However, application of a brief (1 sec) high frequency external stimulus transformed the network, inducing seizure-like events with extracellular K+ increasing up to 8-10 mM and repetitive transitions occurring between tonic and clonic states. This observation was in contrast to results obtained from the intact (without deafferentation) network where the same external stimulation caused only a transient increase in firing rate followed by a return to the baseline after the stimulus was removed. Our study suggests the existence of bistability in the deafferented network, and that the threshold for the spike-wave type of epileptiform activity triggered by external input is reduced following deafferentation and homeostatic scaling. Overall, we show that a model considering interactions between ion dynamics and homeostatic processes can differentiate between interictal paroxysmal activity involving high frequency activity and full-fledged seizure as seen in experimental and clinical settings.

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Poster

248. **Epileptogenesis**

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Topic: C.08. Epilepsy

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Title: The development of an epileptic network following brain injury

Authors: *K. P. LILLIS[1], W. B. SWIERCZ[1], M. A. KRAMER[2], B. J. BACSKAI[1], K. J. STALEY[1];
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Abstract: Following traumatic brain injury, new neurites and synapses form to replace lost connections and restore lost function. If this homeostatic sprouting creates predominantly local connections, a likely result is a recurrently connected neuronal network, prone to generating seizures. In this study, we used the organotypic slice culture model of post-traumatic epileptogenesis (Berdichevsky et al. J Neurosci 2013) to determine the most basic principles by which neurons re-wire following injury. Using genetically encoded calcium indicators and high-frequency two-photon targeted path scan imaging, we recorded calcium signals daily in populations of ≥30 cells to track the spontaneous emergence of synchronous activity in the days leading up to the first seizure. Interestingly, in this severe model of injury, bursts of synchronous activity begin within hours of slice trauma. Using correlation-based network analysis of calcium signals to quantify functional connectivity, we find that statistically significant correlations are initially weak and only exist between nearby cells. As interictal bursting continues, these correlations become progressively stronger and more far-reaching, with correlation strength between pairs of neurons being nearly independent of the distance between them and seizures develop. To more closely examine network changes associated with the initial emergence of synchrony after injury, slices were cultured in a stage mounted miniature incubator and the same 40 cells were imaged hourly for the first 24 hours in vitro. During this time, a similar pattern of initially local and progressively more distal correlations was observed. These results describe the spatial evolution of correlated activity during post-traumatic epileptogenesis. The data support a model in which neurons replace synapses lost to injury by connecting first to neighboring cells, and indicate that the emergence of seizure activity is coincident with the development of longer-range connectivity.

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Poster

248. **Epileptogenesis**

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Topic: C.08. Epilepsy

Support: NINDS Grant 5R01NS034700-21

Title: Potential underlying mechanisms of epileptogenesis after traumatic brain injury

Authors: *Z. WANG, K. STALEY;
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Abstract: Posttraumatic epilepsy is a common long-term consequence of traumatic brain injury (TBI). The underlying mechanisms of the epileptogenesis after TBI are poorly understood.
To investigate the underlying mechanisms of epileptogenesis after TBI, we used the hippocampal organotypic slice culture model of post-traumatic epileptogenesis (Berdichevsky et al. J Neurosci 2013. We combined dual whole-cell patch clamp recording with two photo imaging to study the neuronal activities and networks changes in hippocampal CA1 area during the days in culture after severe slicing trauma. Electrophysiological data were analyzed by custom software (DClamp: available at www.ieeg.org/?q=node/34) to record,identify, separate, and correlate ictal, interictal and postsynaptic activities. Using whole cell patch clamp recording, we found that hippocampal organotypic slices became epileptic after several days of slicing-induced traumatic injury. Interictal spikes occurred earlier than ictal activities, consistent with the chronic EEG telemetry in vivo (White et al Epilepsia 2010) and in vitro field recordings (Berdichevsky et al. Neurobio Dis 2012). After analyzing the ictal activity, interictal activity, and spontaneous postsynaptic currents, we measured the cross-correlation of events between the pair of neurons that we recorded. We found that the cross-correlation of all three activities were greatest locally shortly after slicing, but the correlations of activities in distant pyramidal cell pairs (> 200 um interelectrode distance) increased in the first and second weeks in culture. Two photon imaging of pyramidcal cells in CA1 area after Alexa Fluor 594 Hydrazides was injected through the recording pipette, showed that the dendritic arborization of expanded continuously with days in culture.
Based on above results, we hypothesize that, after severe slicing trauma, neuronal synaptic activities and circuitry undergo continuous modification and rewiring. Increased long-distance correlations in synaptic activities are coincident with the appearance of seizure activity in this preparation, suggesting either that synaptic connections of these distances are necessary for epilepsy, or that ongoing seizure activity favors the survival of these synaptic connections vs. the more local connectivity established earlier.

Disclosures: Z. Wang: None. **K. Staley:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

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Topic: C.08. Epilepsy

Title: Involvement of D3 dopamine receptor and dopamine transporter in epileptogenesis in Genetic Absence Epilepsy Rats from Strasbourg

Authors: F. CAVAREC[1], P. KRAUSS[1], C. BEAUMONT[1], A. BROIZAT[2], J. TOCZEK[2], C. GHEZZI[2], *A. DEPAULIS[1], C. DERANSART[1];
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Abstract: Genetic-Absence-Epilepsy-Rats-from-Strasbourg (GAERS) and Non-Epileptic-Control rats (NEC) derive from an original Wistar-Hannover rat strain (WH). The onset age of spike-and-waves discharges in GAERS is about 25 days post-natal. In adult GAERS with fully developed epilepsy, dopamine plays a modulatory role in seizure expression. Adult GAERS display an over-expression of dopaminergic D3-receptors (D3R) as compared to NEC. Expression and function of D3R and dopamine transporter (DAT) are closely related. The aim of this work was to investigate the putative involvement of D3R and DAT during epileptogenesis by measuring their expression and functionality in GAERS before the onset of epilepsy (P25). D3R expression and functionality was investigated by [125I]-PIPAT autoradiography and quinpirole-induced yawning, respectively, in the three strains at P14 and P21. DAT expression was investigated in GAERS and NEC by [123I]-Ioflupane SPECT imaging in adults and [3H]-GBR12935 autoradiography in adults, P14 and P21 rats. Furthermore, DAT activity was assessed by 3H-dopamine reuptake in synaptosomal living fractions of striatum, cortex and hippocampus of adult rats in the three strains.
Finally, the involvement of D3R was investigated by video-EEG recordings following systemic or intra-accumbens injections of either the D3R agonist (PD128907) or antagonist (SB277011). Autoradiography showed an over-expression of D3R in GAERS in structures known to be involved in seizure initiation (somato-sensory cortex), seizure control (nucleus accumbens,) as well as in other structures (anterior thalamus, olfactory tubercles and islands of Calleja) at P14 and P21, as compared to age-matched NEC and WH. As in adults, this over-expression was associated with a higher number of quinpirole-induced yawns in GAERS at P14 and P21. Neither SPECT imaging nor autoradiographic data revealed any modification in DAT expression between the three strains at any ages. However, we found a consistent increase in 3H-dopamine

reuptake in adult GAERS as compared to NEC and WH in the functional assay.
Both systemic and intra-accumbens administrations of PD128907 increased spike-and-wave discharges, whereas SB277011 had no effect.
Our results suggest that an over-expression of functional D3R already exists before the onset of seizures in GAERS and that, despite a lack of changes in DAT expression, functional changes in this transporter occur in adults. They further support that a profound modification in basal ganglia function together with changes in D3R could be a conditional factor for epileptogenesis.

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Poster

248. **Epileptogenesis**

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Title: The role of adult-generated granule neurons in epileptogenesis and cognitive impairment

Authors: *K. CHO[1,2], N. ITO[3], Z. LYBRAND[2], L. GOOD[4], S. BIRNBAUM[3], S. G. KERNIE[5], H. E. SCHARFMAN[6], A. J. EISCH[3], J. HSIEH[2];
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Abstract: Clinical evidence showing hippocampal pathology and cognitive deficits in patients with temporal lobe epilepsy suggests that neurogenesis may be associated with epileptogenesis and seizure-related memory impairment. Moreover, experimental epilepsy models demonstrate a dramatic increase of hippocampal neural stem cell proliferation and ectopic migration of newborn neurons with hilar basal dendrites and mossy fiber sprouting, which may contribute to spontaneous recurrent seizures (SRS). While previous attempts have been made to examine the role of seizure-generated granule neurons, it is not known if there is a strict cause-and-effect relationship due to the possibility of non-specific side effects of the treatments used to manipulate neural progenitors. Therefore, to investigate the specific role of seizure-induced new neurons in epileptogenesis and cognitive decline, we took advantage of a genetic approach and used a conditional transgenic mouse (Nestin-thymidine kinase) to selectively delete dividing neural stem/progenitors and neuroblasts by ganciclovir (GCV) treatment. Four weeks of GCV administration led to >90% reduction of doublecortin (DCX)-positive cells in the dentate gyrus. Ten-week-old mice were injected with pilocarpine (i.p.) to induce status epilepticus (SE) after GCV treatment. SE was monitored using the Racine scale. Six weeks after SE, the number of DCX-positive cells in the dentate gyrus and the hilus were reduced in GCV-treated mice. Moreover, the frequency of SRS in the GCV group was significantly lower than vehicle-treated mice when examined by 24 hr/day video-EEG from 5 to 7 weeks after SE. Using the novel object location test, which is a well-known paradigm to test hippocampal dependent memory, GCV-treated mice showed recognition of the novel object location whereas vehicle-treated mice did not. Our results suggest that dividing neural progenitors and neuroblasts are a target population for preventing or treating epilepsy in addition to its cognitive comorbidities. The results support the hypothesis that aberrant neurogenesis after seizures contributes to epileptogenesis and cognitive impairment in temporal lobe epilepsy.

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Poster

248. **Epileptogenesis**

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Title: Targeting isoketals to attenuate learning and memory deficits associated with epileptogenesis

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Abstract: Oxidative stress has been identified as a contributing factor to the cognitive decline associated with aging and Alzheimer's disease. Most recently, oxidative stress has been implicated in temporal lobe epilepsy (TLE), however to what degree oxidative stress products contribute to cognitive decline in TLE is unknown. Isoketals (IsoKs) and neuroketals (NeuroKs) are highly reactive gamma-ketoaldehydes formed via the isoprostane and neuroprostane pathways of non-enzymatic, free radical catalyzed oxidation of arachidonic acid and docosahexaenoic acid, respectively. To determine the role of gamma-ketoaldehydes in TLE-associated cognitive dysfunction, we determined 1)hippocampal IsoK and NeuroK adducts by mass spectrometry and 2) the ability of a gamma-ketoaldehyde scavenger to attenuate cognitive dysfunction in a rat model of TLE. Adult male Sprague-Dawley rats were treated with a single, high dose of kainic acid (KA) and monitored for behavioral seizures during status epilepticus and the progression to chronic epilepsy. IsoK, but not NeuroK adducts were increased in hippocampal subregions (dentate gyrus>CA3>CA1) 24 hours following KA. Additionally, IsoK, but not NeuroK adducts were increased in all subregions of the hippocampus 6 weeks after KA. Salicylamine (SA) is an orally active scavenger of IsoKs. A separate cohort of animals was treated with KA as described above, followed by an acute injection of SA, 30 minutes after KA. Seizure intensity and duration was not altered by SA. Animals were allowed to recover and given ad lib access to water supplemented with SA (1g/L) for 7 days (acute) or 6 weeks (chronic). After the 7 day medication period, animals were tested for indices of learning and memory in a novel object recognition task (NOR). The NOR task is uniquely suited for testing KA treated animals at this time point as it is minimally stressful and requires little training of the animal. KA treated animals that received SA performed significantly better than animals not treated with SA (KA vs KA+SA $p= 0.01$), at a level equivalent to control animals (KA+SA vs Control $p=0.15$). Animals in the 6 week treatment group were tested in the standard Morris Water Maze to assess spatial learning and memory. KA animals that received SA performed significantly better than animals that received KA alone on days 4 and 5 of learning (treatment effect $p<0.0001$). Additionally, animals treated with SA had a significantly reduced latency to reach the target quadrant on the probe test for spatial memory (KA vs KA+SA $p < 0.05$) indicative of improved spatial memory. These data suggest that IsoKs are potential mediators of learning and memory deficits associated with experimental TLE.

Disclosures: J. Pearson: None. **L. Liang:** None. **L. Roberts:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vanderbilt University owns a patent for SA licensed to Metabolic Technologies, Inc. in Ames, Iowa that is currently developing SA for treatment of diseases in humans. **M. Patel:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

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Program#/Poster#: 248.18/X16

Topic: C.08. Epilepsy

Title: Pathaphysiology and treatment of epileptic seizures in immature mice following hypoxic/ischemic brain injury

Authors: *J. PENG, C. WU, L. ZHANG;
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Abstract: Hypoxia-ischemia (HI) encephalopathy is a major cause of brain injury and seizure/epilepsy in children. The underlying pathological process is currently not well understood. Effective treatments that lessen brain injury and prevent or reduce subsequent development of epileptic seizures remain to be established. Thus far, one study has described post-HI epileptic seizures in a rat model (Kadam et al, J Neurosci 2010, 30: 404). Our attempt was to replicate the rat model as per the previous study and then to effective treatment strategies. However, we failed to replicate the rat model of HIE under experimental conditions nearly identical to the previous study. We thus used a different approach based on our study of brain ischemia in adult/aging mice. We conducted a reversible model of middle cerebral artery occlusion (rMCAO) in C57 black of 22- 23 day-old. Severe convulsions were observed within 12 hrs post rMCAO but not after sham operation. After treatments with lorazepam and phenytoin, ~70% of seized animals were allowed to survive. These animals ereceived implantation of intracranial recordings at 2 months post rMCAO and then underwent video and EEG monitoring for seizure detection. Of 8 post-rMCAO animals examined intermittently thus far, 6 exhibited interictal- like EEG spikes and 2 exhibited spontaneous seizures during overnight monitoring. Thus, rain ischemia, produced by via the rMCAO model in immature mice or HI episodes in neonatal mice, may induce late-onset epileptic seizures and allow further examination of treatment strategies. We will address these in further experiments. We will first conduct the HI model in neonatal mice. Mouse pups at postnatal day 10 will receive a unilateral occlusion of the common carotid artery and then undergo a hypoxic episode (8% O2 for 90 min). Control animals will receive sham operation without the hypoxic challenge. Post HI animals will receive daily ip injection of memantine (20mg/kg) or saline for 2-3 weeks. If needed, ip injections of diazepam and phenytoin will be applied within 24 hours post HI to control early-onset seizures. All animals will undergo continuous video monitoring (24 hours daily for several weeks, starting from postnatal day 21) to detect spontaneous seizures. After video monitoring,

the animals will undergo magnetic resonance imaging to detect brain injury in vivo. Data from the video monitoring and MRI will reveal animals with spontaneous behavioral seizures and gross brain injury. The animals will then receive implantation of intracranial electrodes and telemetric transmitter and under continuous 24-hour video and EEG.

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Poster

248. **Epileptogenesis**

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Topic: C.08. Epilepsy

Support: Academy of Finland

ERA-Net Neuron

Title: Epileptogenesis in urokinase-type plasminogen activator receptor deficient mice after traumatic brain injury

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Abstract: Objectives. A growing body of evidence has suggested that urokinase-type plasminogen activator receptor (uPAR) contributes to tissue remodeling and plasticity after various epileptogenic insults like traumatic brain injury and status epilepticus. In the present study we investigated consequence of *uPAR* gene deficiency on post-TBI epileptogenesis and co-morbidity.
Methods. The 4 month follow-up study was done on 12 wk old male uPAR (n=26) and Wt (n=20) mice. Traumatic brain injury was induced by controlled cortical impact injury (CCI, velocity 5m/s, depth 0.5 mm) to parietal cortex over the left hippocampus. Motor performance was tested with neuroscore, spatial learning with Morris water-maze, and emotional learning with fear-conditioning to tone and context. At 3 month post-CCI epilepsy phenotype was assessed with a 3-wk continuous video-EEG monitoring and pentyleneterazol (PTZ) seizure-susceptibility test. At 4 month post-CCI mice were pefused for histology.
Results. At 1 wk post-CCI, uPAR-CCI mice performed more poorly in neuroscore than Wt-CCI mice (p=0.05). At 6 wk uPAR-CCI group were also impaired in fear-conditioning to tone and

context as compared to the Wt-CCI group ($p<0.05$). However, spatial memory did not differ between the uPAR-CCI and Wt-CCI groups. At 3 months post-injury, none of mice in the uPAR-CCI and 11% in the Wt-CCI group had spontaneous seizures ($p>0.05$). Spontaneous spiking was detected in 100% of mice in the uPAR-CCI group and in 78 % in the Wt-CCI group ($p>0.05$). In both the uPAR-CCI and Wt-CCI groups the total number of spikes in a 24-h epochs/wk was higher than that in corresponding controls ($p < 0.01$, $p < 0.05$) but there was no difference between the injured groups. Spontaneous epileptiform discharges (EDs) were observed in 43 % of mice in the uPAR-CCI and 11% in the Wt-CCI group ($p>0.05$). In the PTZ test both the uPAR-CCI and Wt-CCI groups had a reduced latency to the first spike ($p<0.01$) and increased total number of spikes ($p<0.01$) as compared to corresponding controls but there was no difference between the injured groups. At 4 months post-CCI, the volume of the cortical contusion was similar in the uPAR-CCI and Wt-CCI mice.
Conclusion. The present study suggests that uPAR deficiency does not alter epileptogenesis after TBI but has an unfavorable co-morbidity modifying effect.

Disclosures: T. Bolkvadze: None. **A. Pitkänen:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 248.20/X18

Topic: C.08. Epilepsy

Support: NIH Grant 5T32NS058280

NIH EUREKA R01NS075429

Title: Abnormal integration of adult-generated granule cells two weeks old at the time of an epilepsy-inducing insult disrupts local dentate microcircuit during epileptogenesis

Authors: *R. T. JONES[1], L. ORMISTON[2], I. MODY[3];
[1]Dept. of Neurobio., UCLA, LOS ANGELES, CA; [2]Neurol., [3]Neurol. and Physiol., UCLA, Los Angeles, CA

Abstract: The dentate gyrus (DG) of the hippocampal formation exhibits significant anatomical reorganization during the epileptogenic process following a precipitating insult (i.e. status epilepticus, SE). Alterations in DG microcircuits include a loss of distinct classes of local interneurons, excitatory mossy cells and the formation of aberrant excitatory connections between DG granule cells (GCs). The DG is one of the few brain regions where neurogenesis

occurs throughout adult life. Progenitor cells in the sub-granular zone of the DG give rise to newborn GCs (NGCs) that develop and integrate into the local DG microcircuit over 6-7 weeks. Previous anatomical studies have demonstrated that precipitating insults, such as SE, disrupt GC integration into the local network. We used retroviral vectors (RV) expressing either G/RFP or channelrhodopsin-2-EYFP (ChR2) to test the hypothesis that abnormally integrated GCs result in functional disruption of the DG microcircuit during epileptogenesis. NGCs were fluorescently labeled with retrovirus in adult C57BL6/J mice two weeks prior to a unilateral cortical kainic acid injection (70 nl, 20 mM) induced SE. Control animals received a saline injection. Following a two-week recovery period (4 wks post-RV), whole-cell recordings were obtained from labeled GCs in coronal brain slices. We find that at two weeks into epileptogenesis, NGCs receive dramatically altered synaptic connectivity despite exhibiting intrinsic excitability comparable to controls. When ChR2 was expressed in NGCs, voltage-clamp recordings from nearby ChR2-negative GCs revealed that a brief (50 ms) photoactivation results in a significant increase in EPSC frequency and amplitude that persists for up to 30 seconds post-ChR2 activation. Our data demonstrate an enhanced synaptic connectivity of NGCs during epileptogenesis and provide evidence that their disrupted integration is sufficient to generate abnormal activity within the epileptic DG microcircuit.
Supported by NIH 5T32NS058280 to R.T.J., and EUREKA R01NS075429 and the Coelho Endowment to I.M.

Disclosures: R.T. Jones: None. **L. Ormiston:** None. **I. Mody:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 248.21/Y1

Topic: C.08. Epilepsy

Support: ISU start-up funds, USA.

BBSRC, UK

Title: Refined kainate C57BL/6J mouse model of epileptogenesis

Authors: S. PUTTACHARY[1], E. BEAMER[2], K. TSE[2], G. SILLS[3], *T. THIPPESWAMY[1]; [1]Dept. of Biomed. Sci., Iowa State Univ., Ames, IA; [2]Inst. of Ageing and Chronic Dis., [3]Univ. of Liverpool, Liverpool, United Kingdom

Abstract:
The development of a refined kainate (KA) C57BL/6J mouse model of status epilepticus (SE) and epileptogenesis by a repeated low-dosing (RLD) of KA (5 mg/kg, i.p; at 30min interval) is compared with an established single-high dose (SHD) of KA (20 mg/kg, i.p) model. In RLD group, a greater inter-animal uniformity, increase in the duration of convulsive motor seizure (CMV, Racine scale ≥3) with a great advantage of a significant reduction in the mortality rate were achieved when compared to the SHD. EEG analysis of 2h duration from the point of first observed CMS to diazepam treatment and up to 7days post-KA showed consistently higher spike frequency, except between 38 and 58h, in RLD group. Neuronal activation marker, c-Fos expression in the hippocampus was widespread with 3-3.5 fold increase in CA3 and CA1 region in RLD mice, when compared with SHD group, at 2h after the onset of first CMS. Both groups had gliosis at 7d post-KA. In RLD group the reactive
astrocytes and microglia dominated amongst the dispersed CA3 pyramidal cells and the hilus of the dentate gyrus, and NeuN immunoreactivity demonstrated marginally decreased number of hilar cells when compared to SHD group. Cresyl violet did not reveal morphological changes in either group. However, fluorojade-B staining showed greater number of positive cells in CA3 and, surprisingly, in dentate granule cells and the hilus. Timm staining at 7d post-KA revealed distinct staining in the supragranular layer of the dentate gyrus in RLD group, while it was mild in SHD mice and absent in control. Overall these results from integrated behavioral, electrographic, histological and immunohistochemical analyses confirm that RLD of KA is a reliable and better mouse model of SE and epileptogenesis than a SHD model of KA.

Disclosures: S. Puttachary: None. **E. Beamer:** None. **K. Tse:** None. **G. Sills:** None. **T. Thippeswamy:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 248.22/Y2

Topic: C.08. Epilepsy

Title: Dynamic changes in kainate receptor mediated responses in hippocampal area CA3 during epileptogenesis in the rat

Authors: *T. MODEBADZE, G. WOODHALL;
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Abstract: Adaptations in neuronal network function in response to destabilizing influences are the subject of much current research, however, changes underlying the establishment of epilepsy in temporal lobe networks remain poorly characterised. We investigated spontaneous and kainate-induced oscillatory neuronal network activity in a model of chronic epileptogenesis in the juvenile rat. Epileptogenesis was induced using a low-dose lithium-pilocarpine-xylazine (LPX) model, and 450 μm thick slices were made at 3 time points: 24h post-induction, at 4-6 weeks during the latent period and at 3 months following the development of spontaneous recurrent seizures. Local field potential (LFP) recordings were made in slices containing hippocampal area CA3 in epileptic and age-matched control (AMC) animals. In almost all slices, spontaneous oscillatory activity could be recorded in the gamma (30-80 Hz) and/or very fast oscillation (VFO; >150 Hz) bands. At 24 hours post induction, spontaneous gamma activity and VFO were prominent features in area CA3 of lesioned rats, while much less VFO activity was seen in controls. When we applied 100 nM kainic acid to slices at 24 hours post-induction, mean peak gamma power increased by 3198.4 ± 1333 μV^2 and this was similar in AMC data. During the latent period, however, whilst spontaneous activity remained present, the response to kainate was depressed, such that challenge with 200 nM kainate elicited only a 267.4 ± 259.3 μV^2 increase in peak gamma power compared to 840.4 ± 371 μV^2 in controls. Interestingly, at this time point seizure-like activity was extremely difficult to evoke, even using higher concentrations of kainate. Following the expression of behavioural seizures, spontaneous activity in area CA3 was characterised by florid VFO (150-800 Hz) and challenge with 100 nM kainic acid resulted in a significant increase in mean peak gamma power similar to controls (1140 ± 184.8 μV^2) and often elicited ictal-like events. These data suggest that during the latent period, the destabilizing influence of seizure activity is mitigated through altered network excitation, perhaps mediated by a reduction in kainate receptor expression.

Disclosures: T. Modebadze: None. **G. Woodhall:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 248.23/Y3

Topic: C.08. Epilepsy

Support: NC3Rs

Title: Low-dose lithium-pilocarpine-xylazine epileptogenesis - a refined model of chronic epilepsy in the rat

Authors: *G. WOODHALL, N. MORGAN;
Aston Univ., Birmingham, United Kingdom

Abstract: Epileptogenesis in the rat has provided a wealth of data concerning alterations in intrinsic and synaptic function underlying the establishment of epileptogenic neuronal circuits, however, many models of epilepsy show high rates of mortality. In addition, Sloviter (2005) has raised the potentially confounding issue of global cortical damage related to long-duration *status epilepticus* and questioned whether such procedures encompass vascular, as well as neuronal pathology. The refined lithium-pilocarpine-xylazine (LPX) model make use of an animal-specific titration of pilocarpine dose, coupled with early intervention (Racine stage 4) by intramuscular injection with xylazine to produce seizures that do not progress uncontrollably, and which are terminated fully after 60 minutes using a multi-drug cocktail acting at NMDA, GABA-A and mGluRs. This method provides tractable and repeatable seizure generation and reduces risk of uncontrolled generalization. Following initial recovery, mortality rates are 0-4% within groups, and epileptogenicity at 67-84% is comparable to other models. Animals typically undergo a quiescent period of 7-10 weeks prior to the onset of spontaneous recurrent seizures (SRS), which vary in frequency from several per day to low numbers per week, a feature that mimics variability in human disease. Initial investigations using the *in vitro* brain slice preparation in animals at various time points following epileptogenic insult with the LPX model show spontaneous neuronal network activity, including VFO (200-600 Hz), beta (15-29 Hz) and gamma (30-80 Hz) oscillations in area CA3 of hippocampus and slow-wave-burst complexes in layer II of the medial entorhinal cortex (mEC). These data suggest strongly that neuronal networks are hyperexcitable, but essentially intact, and few ictal-like events are seen. The patterns of activity recorded *in vitro* using the LPX model closely mimic events recorded in this laboratory using slices of human brain tissue resected from paediatric patients undergoing treatment for intractable epilepsy at Birmingham Children's Hospital, suggesting that the model shares at least some features with human disease. Further experiments have shown that even moderate increases in the duration of the initial insult show a much different pattern of activity to that described above, with little spontaneous rhythmicity and a prevalence of complex, long duration epileptiform discharges in CA3 and mEC. These data suggest the existence of a clear window, within which epileptogenicity can be maintained in intact networks and beyond which activity is indicative of more widespread neuronal damage.

Disclosures: G. Woodhall: None. **N. Morgan:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 248.24/Y4

Topic: C.08. Epilepsy

Support: Elite Network of Bavaria

Title: Impact of the endocannabinoid and endovanilloid system on the generation of a hyper-excitable neuronal network

Authors: *E.-L. VON RÜDEN[1,2], M. JAFARI[2,3], R. M. BOGDANOVIC[2,4], L. VILELA[5], C. T. WOTJAK[6], H. POTSCHKA[1];
[1]Inst. of Pharmacology, Toxicology, and Pharm., Munich, Germany; [2]Grad. Sch. of Systemic Neurosciences, Munich, Germany; [3]Inst. of Clin. Neuroimmunology, Munich, Germany; [4]Inst. of Pharmacology, Toxicology and Pharm., Munich, Germany; [5]Inst. of Biol. Sci., Minas Gerais, Brazil; [6]Max Planck Inst. of Psychiatry, Munich, Germany

Abstract: The endocannabinoid (EC) system serves a key function in regulating neuronal activity throughout the central nervous system.
The EC anandamide not only activates cannabinoid type 1 (CB1) receptors on presynaptic terminals of several neuronal populations, but also transient receptor potential vanilloid (TRPV1, formerly vanilloid receptor) channels which are nonselective cation channels. Whereas activation of CB1 leads to a decrease in neurotransmitter release, TRPV1 channels enhance glutamate release.
Given their role in balancing excitatory and inhibitory transmission, both the EC and the endovanilloid (EV) systems can be considered as putative targets in central nervous system diseases with unrestricted neurotransmission, including epilepsies. In this study, we modulated the EC/EV system both pharmacologically and genetically and analyzed the impact on kindling progression in mice.
The following mouse strains were used for pharmacological and genetic modulation of the EC/EV system: NMRI mice, conditional CB1-receptor knockout (KO)- mice, with selective deletion of CB1 from principal neurons (CamK-CB1-KO) or GABAergic neurons of the forebrain (GABA-KO) and conventional TRPV1-KO mice. All strains were backcrossed to the C57BL/6N strain. Experiments were performed with corresponding wildtype controls. Male mice were implanted with a stimulus and recording electrode into the right basolateral amygdala. The mice were electrically stimulated once daily. NMRI mice received i.p. injections of either the selective CB1 receptor-antagonist rimonabant (5 mg/kg i.p.), the selective TRPV1 antagonist SB366791 (1 mg/kg i.p.) or vehicle 30 minutes prior to each kindling stimulation. Six to twelve animals were used per group.
Pharmacological antagonism of CB1 and the absence of CB1 from principal neurons of the forebrain failed to affect the development of generalized seizures. The mean number of stimulations needed to reach a generalized stage five seizure did not differ between the groups. However, both pharmacological and genetic interventions caused longer seizure duration. The

opposite was achieved when CB1 was deleted from GABAergic neurons, which resulted in shorter seizure duration. Initial seizure thresholds proved to be lower in naïve CamK-CB1-KO mice as compared to controls. No effects were seen upon pharmacological and genetic modulation of the EV system.
The study indicates that EC but not EV signaling affects seizure susceptibility as well as endogenous termination of seizure activity, without influencing seizure severity over time. This is dependent on the neuronal subpopulation being modulated.

Disclosures: E. Von Rüden: None. **M. Jafari:** None. **R.M. Bogdanovic:** None. **L. Vilela:** None. **C.T. Wotjak:** None. **H. Potschka:** None.

Poster

248. **Epileptogenesis**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 248.25/Y5

Topic: C.08. Epilepsy

Support: Deutsche Forschungsgemeinschaft

BMBF

Else Kröner-Fresenius-Stiftung

Individuelle Graduiertenförderung der Universität Bonn

BONFOR

Title: LIM-domain-binding proteins interaction in neuronal development and epileptogenesis

Authors: *B. K. IWANIUK[1], A. GROTE[2], K. VAN LOO[1], S. SCHOCH[1], A. J. BECKER[1]; [1]Neuropathology, [2]Neurosurg., Univ. Bonn Med. Ctr., Bonn, Germany

Abstract: Recent data suggested LIM-domain-binding 2 (LDB2) expression to be strongly reduced in the most frequent epilepsy-associated glioneuronal brain tumors, i.e. gangliogliomas (Fassunke et al., Brain 2008). LDB proteins are multi adapter proteins that interact with different transcription factors and kinases like the STE-20 like kinase (SLK). LDBs and SLK are strongly expressed during developing brain.
Here, we examined in detail the functional interplay of LDB1 and -2 with SLK in brain development and potentially aberrant patterns in the emergence of cortical malformations. shRNA-mediated silencing of mouse LDB1, LDB2 or mouse SLK in primary neurons resulted in

aberrant morphology affecting axons and dendrites *in vitro*. Overexpression of the human LDB2 isoform or of human SLK rescued the phenotype of altered neuronal arborization. Furthermore double knock down of LDB1 and LDB2 could not be rescued by SLK overexpression indicating the necessity of LDB-SLK complexes for regular neurite growth *in vitro*. Currently, mice embryos (E14) are subjected to intraventricular *in utero* electroporation (IUE) with shRNAs targeting mouse LDB1 and 2 isoforms. Our first results indicate adult mice with IUE of LDB2 shRNAs to have neurons with aberrant morphology, i.e. potentially reflecting the irregular neuronal component of gangliogliomas.
LDBs and SLK represent key factors of neuronal development, for which our current data suggest fine tuned interactions and a potential functional impact in epilepsy-associated tumor pathogenesis, i.e. an aspect we currently analyze in IUE models more in detail.

Disclosures: B.K. Iwaniuk: None. **A. Grote:** None. **K. van Loo:** None. **S. Schoch:** None. **A.J. Becker:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.01/Y6

Topic: C.21.Perinatal Brain Injury

Support: FAPERGS

CNPq

CAPES

UFRGS

Title: Benefits of early exposure to enriched environment on motor behavior and on the spinal cord plasticity in a cerebral palsy rat model

Authors: *S. MARCUZZO[1,2], M. R. MARQUES[3], F. STIGGER[3], O. A. AUGUSTIN[3], E. SEGABINAZI[3], S. BARBOSA[3], F. V. PIAZZA[3], M. ACHAVAL[3];
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Abstract: Cerebral Palsy (CP) results from nonprogressive lesions in the immature brain. The secondary impairment associated with CP normally is accompanied by morphologic,

biochemical and physiological changes in the neuromuscular system. Rehabilitation in CP is recommended throughout early childhood while the normal neuromotor development can be facilitated. In experimental studies, the environmental enrichment (EE) may be considered a rodent correlate of therapeutic interventions in humans. It provides physical activity, learning experiences, increased somatosensorial inputs and social interaction.
The aim of this study was to verify the effects of the early EE on balance and gait in a CP rat model and investigate possible biological substrate involved in it.
All procedures were approved by the Ethical Committee at the Universidade Federal do Rio Grande do Sul, Brasil (23594). The CP model was induced with the association of maternal exposure to low doses of bacterial endotoxin (lipopolysaccharide), perinatal anoxia and sensorimotor restriction of the pups. The pups were divided in control group (CT group), animals exposure to EE (EE group), CP model (CP group) and CP exposure to EE (CP-EE group).
At 29 days of life, the motor balance was assessed using a Rotarod and the walking pattern consisted in measure the hind paw stride length. After motor testing, the rats were euthanized by transcardiac perfusion and the spinal cord segments were removed. To motoneuronal analysis, the cross-sectional area (CSA) of the motoneurons in ventral horn of the spinal cord was estimated by the point-couting technique. To immunoistochemical procedure, the intensity of the synaptophysin immunoreaction was measured in the ventral horn of the spinal cord. The data were analyzed by Two-way analysis of variance, followed by post hoc Tukey.
The CP-EE group showed better performance on motor balance when compared to CP ($p = 0.04$). The CP group had a shorter stride length compared to CT ($p = 0.0005$) and EE ($p = 0.0004$); however, CP-EE improved the stride length when compared to CP ($p = 0.004$). The mean size of motoneuronal somata showed an increase in CP-EE group in relation to CP ($p = 0.04$) and CP-EE group had more synaptophysin immunoreactivity in ventral horn of the spinal cord when compared to CP ($p = 0.02$).
The increase of the stimulus provided for EE prevented motor deficits in a cerebral palsy rat model. The increase in CSA of the motoneurons and the synaptophysin immunoreaction in ventral horn of the spinal cord could be possible biological substrate involved in these effects. These results emphasize the importance of the early therapeutic intervention in CP to prevent secondary impairment.

Disclosures: S. Marcuzzo: None. **M.R. Marques:** None. **F. Stigger:** None. **O.A. Augustin:** None. **E. Segabinazi:** None. **S. Barbosa:** None. **F.V. Piazza:** None. **M. Achaval:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.02/Y7

Topic: C.22.Stroke Recovery

Support: Seed Grant UMB: McCombe Waller

Title: A loud acoustic stimulus evoked- response during movement planning facilitates voluntary reach in patients with chronic stroke

Authors: C.-L. YANG, *S. MCCOMBE WALLER, A. HOWE, M. W. ROGERS;
Dept. of Physical Therapy and Rehabil. Sci., Univ. of Maryland, Sch. of Med., BALTIMORE, MD

Abstract: Objective: Delivering a loud acoustic stimulus (LAS) during the preparation phase of an intended action can trigger preplanned movement with earlier movement onset and comparable movement characteristics as the voluntary movement. Our previous work found a marked reduction in the incidence of LAS-evoked responses in stroke subjects compared to controls during the planning period for both anticipatory postural adjustments and the goal intended reach in standing. However the impact of an altered LAS-evoked response on cued voluntary movement after stroke has not been examined. We investigated the influence of LAS presented at 200 ms prior to a go cue on timing of a voluntary reach in standing for individuals with stroke. **Subjects:** Eight participants with chronic ischemic stroke. **Methods:** Using a simple reaction time paradigm, subjects were standing on separate force platforms and received a warning light cue to "get ready to reach" followed 2000 ms later by a go light cue to "reach as quickly as you can" with the paretic arm to touch a target ball. In 1/3 of the trials we applied LAS of 124 dB at 200ms prior to the "go" cue. We selected this time as it represents the point at which a movement is normally almost fully prepared. For the voluntary cued movement, we compared the timing of reach onset and reach onset to peak displacement in LAS trials that did and did not result in an LAS- evoked response. We further compared this to the reach onset and onset to peak timing during cued reaching with no LAS (control reach). **Results:** Of the eight participants three subjects presented with LAS trials that did and did not result in an LAS-evoked response. In this subset of three subjects, trials in which an LAS-evoked movement at 200ms prior to the go cue was elicited, the voluntary reach demonstrated earlier movement onset and faster onset to peak compared to the trials in which there was no LAS-evoked movement and our control trials where no LAS was introduced. **Conclusion:** There is a marked reduction in LAS-evoked responses in subjects with stroke that may indicate suppression of ascending brainstem pathways potentially by corticoreticular inputs. When LAS-evoked responses are present, the ascending pathways may be activated, which in turn may result in increased inputs to motor cortex and therefore facilitation of a faster voluntary reach. The altered ability to achieve LAS-evoked responses may represent variability in the state of preparation as a result of stroke.

Disclosures: C. Yang: None. **S. McCombe Waller:** None. **A. Howe:** None. **M.W. Rogers:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.03/Y8

Topic: C.22.Stroke Recovery

Support: Dept of Veterans Affairs RR&D REAP

Title: Promoting recovery after stroke using botulinum toxin for limb immobilization

Authors: *A. ORR, D. BINGHAM, M. KAWABORI, J. KIM, Z. ZHENG, T. LAM, S. MASSA, J. LIU, M. YENARI, R. SWANSON;
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Abstract: Motor rehabilitation strategies after stroke aim to improve function of the paretic limb. Evidence suggests that immobilization of the healthy limb can promote functional recovery of the paretic limb following stroke involving motor cortex. In rodents this has be modeled using casts, harnesses, and other means of restricting use of the non-paretic forelimb after middle cerebral artery stroke. Here we evaluated an alternative approach, using botulinum toxin injections to limit function of the non-paretic forelimb. Adult male rats were subjected to permanent ligation of the left distal middle cerebral artery, resulting in a paretic R forelimb, or sham surgery alone. The rats were then randomized to one of 3 treatment groups: 1) no treatment; 2) botulinum toxin injections on day 1; or 3) cast placement on day 2. All rats were subsequently given “physical therapy” consisting of 60 minutes / day of assisted exercise on climbing nets, roller balls, and rope climbing. Botulinum toxin injections were made into the left (non-paretic) forelimb. The forelimb was injected with 10 units of botulinum toxin A, distributed over 8 sites in both the ventral and dorsal compartments of the distal and proximal limb. Weakness in the injected limb became apparent after 24 hours. Casting was accomplished by wrapping ipsilateral limb in felt and positioning against sternum with elastic wrap and plaster, and was left in place for 3 weeks. Rats with bilateral forelimb impairment due to either stroke plus casting or stroke plus botulinum injections were able to feed and groom normally. However, ambulation was impaired, and the rats were less able to participate in physical therapy than rats treated with either botulinum alone or stroke alone. Nevertheless, both groups showed modest improvement over the stroke only group with respect to function on the cylinder test and foot-fall tests when assessed 3 months after stroke. The botulinum toxin approach to limb paresis had both advantages and disadvantages over traditional physical limb immobilization. The major advantage was that it was far less stress-inducing to the subject animals. A disadvantage was that

the paresis took roughly 10 weeks to resolve, and any degree of residual paresis confound interpretation of the behavioral assessments.

Disclosures: A. Orr: None. **D. Bingham:** None. **M. Kawabori:** None. **J. Kim:** None. **Z. Zheng:** None. **T. Lam:** None. **S. Massa:** None. **J. Liu:** None. **M. Yenari:** None. **R. Swanson:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.04/Y9

Topic: C.22.Stroke Recovery

Title: Protection by LOXBlock-1 in novel mouse models of permanent and transient focal ischemia: Inhibiting 12/15-lipoxygenase to treat stroke

Authors: *H. KARATAS-KURSUN[1,2], Y. LIU[2], E. LO[2], K. VAN LEYEN[2];
[1]Hacettepe University, Inst. of Neurolog. Sci. and Psychiatry, Ankara, Turkey; [2]Massachusetts Gen. Hospital, Harvard Med. Sch., Neuroprotection Res. Laboratory, Dept. of Radiology, Charlestown, MA

Abstract: Stroke damages the neurovascular unit and causes massive cell death during the acute phase. 12/15-LOX contributes to brain damage after middle cerebral artery occlusion (MCAO), and is increased in humans following a stroke. The aim of this study was to investigate the effects of the 12/15-LOX inhibitor LOXBlock-1 (LB1) in a mouse permanent distal MCAO model induced by FeCl3, and in conjunction with subsequent thrombolysis with tPA, the only drug with current FDA approval for acute stroke treatment.
We topically applied 30% FeCl3 to induce permanent distal MCAO in C57Bl6 mice. LB1 (50 mg/kg) or vehicle (DMSO) treatments were given intraperitoneally (ip) 2 hours after MCAO. Regional cerebral blood flow was monitored by laser-Doppler flowmetry. A neurological severity score (NSS) test was performed before ischemia and 24 h after MCAO. Outcomes were NSS result, weight loss and infarct volume.
To study tPA-induced reperfusion, we initiated thrombosis with 10% FeCl3 in CD1 mice. 4 hours after onset of ischemia either LB1 (50 mg/kg) or vehicle was injected ip and at the same time, iv tPA (10 mg/kg, the dosage commonly used in rodents) administered for reperfusion. NSS was performed before ischemia and 24 h after MCAO. Outcomes were NSS result, weight loss, infarct and hemorrhage volume, and reperfusion rate.
FeCl3 induced distal MCAO caused increased LOX immunoreactivity in the ischemic cortex,

along with an increase in apoptosis-inducing factor AIF. LB1 treatment, applied 2 hours after ischemia, significantly decreased infarct volume at 24 h of permanent distal MCAO (n=6/group). Weight loss was also significantly better in LB1 treated group. NSS results were in favor of LB1 group but it was not significant.
Distal MCAO induced by 10 % FeCl3 and treatment with 10 mg/kg tPA plus LB1 or vehicle 4 hours after onset of ischemia showed that tPA plus LB1 treatment significantly decreased infarct and hemorrhage volume, compared to tPA alone. Successful reperfusion was achieved more frequently in the LB1 treated group (5/8) than in the vehicle group (2/9). NSS results and weight loss were improved in LB1 treated group, but differences were not significant.
LB1 treatment, applied 2 hours after ischemia, significantly decreases infarct volume and NSS values in permanent distal MCAO. LB1 treatment applied 4 hours after ischemia and reperfused with tPA, significantly decreases infarct volume and hemorrhage volume. Distal MCAO with FeCl3 model may be useful to screen drugs in short-term recovery phase. LB1 provides significant treatment effects over several models of stroke and represents a promising new approach to stroke therapy, either alone or in conjunction with tPA treatment.

Disclosures: H. Karatas-Kursun: None. **Y. Liu:** None. **E. Lo:** None. **K. van Leyen:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.05/Y10

Topic: C.22.Stroke Recovery

Support: Industry sponsored study O'Brien Technologies, Inc.

Title: Frontal-subcortical function and use of assistive speech devices for aphasia

Authors: *A. CHAUDHARI[1,3], J. S. MANISCALCO[3,4,5,6], J. ZHANG[3], D. S. WILLIAMSON[7], U. ADLER[8], A. M. BARRETT[2,3,8,9];
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Abstract: Objective: To identify neuropsychological predictors of successful assistive device use in aphasia. **Background:** Assistive speech devices (ASDs) may augment communication efficacy in aphasia.However, patients may have difficulty using a mobile device, which requires

diverse mental abilities including cognitive motor planning, concentration, visuospatial orientation and sequencing. We wished to evaluate whether performance on specific neuropsychological tests could predict device use success, thereby assisting clinicians in identifying ASD candidates. **Methods:** 20 people with aphasia (60.6 ± 14.3 years) completed 9 neuropsychological tasks, including Finger Tapping Test (FTT), aphasia-adapted Frontal Assessment Battery (FAB), Test of Oral and Limb Apraxia (TOLA), Western Aphasia Battery (WAB), Behavioral Inattention Test (BIT), Communicative Effectiveness Index (CETI), Catherine Bergego Scale (CBS), Naturalistic Action Test (NAT), and Neuropsychological Assessment Battery (NAB). All patients were trained to use an ASD (O'Brien Technologies Survivor Speech Companion System) followed by 7 days' unrestricted home use. Then, each participant completed 3 device-based communication tasks (use the device to tell your marital status, give directions to Kessler Foundation, and ask for a glass of water). We performed a Stepwise Discriminant Function Analysis to evaluate how pre-trial tests performed in grouping High (>80%) vs. Low (<80%) scorers at device-based communication. We also conducted a factor analysis to explore the underlying structure of the groups' neuropsychological test scores. **Results:** The Frontal Assessment Battery (FAB) was the only significant predictor of device use success (Wilk's Lambda=0.713, F=6.856, p=0.018, 28.7% variance), correctly classifying 80% of High/Low scores. A Factor Analysis indicated that a factor including the FAB, WAB, BIT, NAB and TOLA explained 45% of variance in overall neuropsychological performance (eigenvalues>1). **Conclusions:** Identifying people with aphasia who can benefit from an assistive speech device is vital to prescribing an ASD. Here, aphasia severity was *not* predictive, but an aphasia-adapted version of the Frontal Assessment Battery predicted device success. These results show that frontal cognitive assessment may be needed in standard ASD assessment. Further research to identify which skills assessed by the FAB best predict device use success, and their relation to other neuropsychological deficits in aphasia, are indicated.

Disclosures: A. Chaudhari: None. **J.S. Maniscalco:** 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.; O'Brien Technologies Inc., Kessler Foundation. **J. Zhang:** None. **D.S. Williamson:** None. **U. Adler:** None. **A.M. Barrett:** 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.; O'Brien Technologies Inc., Kessler Foundation, NIH Department of Education.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.06/Z1

Topic: C.22.Stroke Recovery

Support: Heart and Stroke Foundation of British Columbia and the Yukon Grants-in-Aid

Title: Evaluating the relationships between hand function with measures of brain structure and function in chronic stroke

Authors: *M. R. BORICH[1], E. DAO[1], J. D. EDWARDS[2], S. K. MEEHAN[3], L. A. BOYD[1]; [1]Physical Therapy, [2]Sch. of Population and Publ. Hlth., Univ. of British Columbia, Vancouver, BC, Canada; [3]Kinesiology, Univ. of Michigan, Ann Arbor, MI

Abstract: Stroke is the leading cause of adult disability and less than half of individuals regain full arm function. Transcranial magnetic stimulation (TMS) techniques can be used to gain insights into brain neurophysiology. Neuroimaging can provide information about brain anatomy that may be used in conjunction with TMS to identify new biomarkers of recovery after stroke. Here, we aimed to characterize the relationship between TMS measures of transcallosal interhemispheric cortical excitability, brain structure, and manual dexterity in chronic stroke. Thirty-four individuals with chronic (>6 months) stroke (mean age: 65.6±7.4 years, 8F) underwent a TMS assessment, magnetic resonance imaging scanning session, and manual dexterity testing (Box and Blocks Test or BBT). The magnitude of transcallosal inhibition (TCI) elicited by TMS applied over primary motor cortex during active ipsilateral hand contraction was used to index interhemispheric cortical excitability. Precentral gyral thickness and corpus callosum volume were used as metrics of brain structure and extracted using the Freesurfer image analysis suite. Performance on the BBT was defined as the number of blocks transferred in one minute. One-way analyses of variance were performed to evaluate hemispheric differences in TCI magnitude and cortical thickness and differences between paretic and non-paretic hand dexterity. Correlational analyses were conducted to characterize relationships between interhemispheric excitability, precentral gyral thickness, callosal volume, and hand dexterity.

Results demonstrate significant differences in TCI ($p=0.01$) and precentral gyral thickness ($p=0.001$) between lesioned and non-lesioned hemispheres and in BBT performance between hands ($p<0.001$). BBT performance was positively correlated with precentral gyral thickness ($r=.55$, $p<0.001$). Magnitude of TCI was linked to central callosum volume ($r=-.45$, $p=0.006$). No other significant correlations were observed.

Differences in interhemispheric cortical excitability were observed in individuals with chronic stroke, as reflected by greater TCI during stimulation of the ipsilesional primary motor cortex. Reduced dexterity was demonstrated in the paretic hand. Increasing TCI magnitude was correlated with larger central callosum volume. Higher levels of hand dexterity were correlated with greater precentral gyrus thickness. These results suggest changes in brain neurophysiology

and anatomy may be correlated and related to hand function in individuals with chronic stroke. These findings may be used in future work to evaluate and predict response to rehabilitation.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

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Program#/Poster#: 249.07/Z2

Topic: C.22.Stroke Recovery

Support: Florida Biomedical Grant 3KN01

Title: Probabilistic tractography of transcallosal motor tracts in the chronic phase after stroke

Authors: *D. B. ARCHER, G. MISRA, C. PATTEN, S. COOMBES;
Univ. of Florida, Gainesville, FL

Abstract: Following stroke, the integrity of white matter tracts can be affected both locally and distally to the primary lesion location. Diffusion magnetic resonance imaging (MRI) can provide unique and detailed information about white matter integrity. Although previous observations have demonstrated that inter-hemispheric interactions are altered after stroke, potential deficits in the structural integrity associated with these interactions remains poorly understood. Of particular interest are the motor fibers that link the corpus callosum to the primary motor cortex (M1). A reduction in the integrity of M1-callosal motor fibers was recently demonstrated, but the authors interpreted their findings with caution because they used a low-resolution scan with non-isotropic voxels and 25 diffusion directions. The goal in the current study was to examine the integrity of the M1-callosal tracts in the chronic phase of stroke using 2x2x2 resolution 64-direction diffusion MRI. Left and right M1 regions were isolated from the human motor area template and were transformed from standardized space to native subject space to create M1 seed regions. Probabilistic tractography was used to identify the tracts that link M1to the corpus callosum in each hemisphere. Analyses were performed in native subject space. Directional diffusivity values of the callosal tract in each hemisphere were obtained. A contralesional/ipsilesional ratio was then calculated between hemispheres, with a value of one reflecting similar integrity between hemispheres, and a value greater than one reflecting an ipsilesional deficit. Fugl-Myer assessments were collected. Maximum voluntary contractions (MVC) of each hand were also recorded and a corresponding ratio was calculated. We report four findings. 1) MVC of the impaired hand and MVC ratios between hands revealed force

deficits in the stroke as compared to control group. 2) Tract volume ratios were higher in stroke patients compared to controls. 3) Structural integrity in the ipsilesional M1-callosal motor tract was reduced after stroke as indexed by increases in the mean and variability of perpendicular diffusivity and parallel diffusivity. 4) Mean diffusivities and ratios of diffusivities correlated with Fugl-Myer and MVC scores, demonstrating that deficits in the structural integrity of M1-callosal tracts relate to deficits in motor function. Our findings replicate and extend previous studies which have linked the integrity of callosal motor fibers to function in the chronic phase after stroke.

Disclosures: D.B. Archer: None. **G. Misra:** None. **C. Patten:** None. **S. Coombes:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

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Program#/Poster#: 249.08/Z3

Topic: C.22.Stroke Recovery

Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20110014021)

a KOSEF grant (M10644000022-06N4400-02210)

Title: Influence of BDNF genotype to the response of rTMS modes in chronic stroke patients

Authors: *W. CHANG, K. UHM, J. HWANG, H. SHON, Y.-H. KIM;
Physical Med. and Rehabil., Samsung Med. Ctr., Seoul, Korea, Republic of

Abstract: Objective: Brain-derived Neurotrophic Factor (BDNF) polymorphism was known to influence on the response of repetitive transcranial magnetic stimulation (rTMS). In Asian population, especially Korean, a higher incidence of the BDNF polymorphism was reported than the western. This study aims to investigate the different response to rTMS modes according to the BDNF genotype in stroke patients. **Methods:**
Nineteen patients (9 males, mean age 57.1) with chronic stroke. All participants received randomly arranged 3 rTMS modes over the non-dominant M1 with more than 24 hrs of washout period; 1st condition; 10 Hz rTMS with sub-threshold intensity (90% of rMT), 2nd condition; 10 Hz rTMS with supra-threshold intensity (110% of rMT), and 3rd condition; sham rTMS. Cortical excitability was assessed using amplitude of the motor-evoked potentials (MEPs) before and after rTMS. Hand function was also assessed using the Box and Block test. Data were grouped and analyzed according to the BDNF polymorphism; Val/Val group vs. Met allele group.

Results: Five (26.3%) and 14 (73.7%) patients were classified into Val/Val and Met allele groups, respectively. Before rTMS, there was no significant difference in age, type and duration of stroke, MEP amplitude, and hand function between two groups. In each Val/Val and Met allele group, MEP amplitudes were significantly increased after applying sub- and supra-threshold rTMS (the 1^{st} and the 2^{nd} conditions, $p<0.05$), but not in the sham condition. However, there was no statistically significant difference in MEP amplitude changes between the 1^{st} and 2^{nd} conditions. The hand motor function showed significant improvement in only 1^{st} condition ($p<0.05$) in both Val/Val and Met allele groups without significant difference between two groups. **Conclusion:** This study revealed that high-frequency rTMS with both sub- and supra-threshold resulted in enhanced cortical excitability and improvement of hand function in patients with chronic stroke irrespective of BDNF polymorphism. In contrast with healthy subjects, high-frequency rTMS with the supra-threshold intensity over M1 did not demonstrate enhancement effect on the cortical excitability changes compared to the sub-threshold rTMS in stroke patients in both groups. Therefore, rTMS with sub-threshold intensity can be used as an intervention of enhancing cortical excitability in stroke patients irrespective of BDNF polymorphism (Supported by
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Disclosures: **W. Chang:** 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.; Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology and a KOSEF grant. **K. Uhm:** None. **J. Hwang:** None. **H. Shon:** None. **Y. Kim:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Topic: C.22.Stroke Recovery

Support: NIH Grant T32HD007434

NIH Grant R01HD068290

NIH Grant 5K08NS064365

Title: Resting-state functional connectivity in a somatomotor network and its association with the continuum of upper extremity behavioral recovery in chronic stroke

Authors: *M. URBIN[1], X. HONG[2], C. LANG[2], A. CARTER[2];
[1]Washington Univ. Sch. of Med., MO; [2]Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Stroke disrupts the connectivity between functionally related brain regions that are both adjacent to and remote from the lesion site. One neuroimaging technique used to investigate connectivity between motor-related brain regions involves measuring the temporal synchrony of low-frequency (<0.1 Hz) fluctuations in the fMRI BOLD signal during resting wakefulness. Recent findings indicate that the coherence of this intrinsically generated brain activity (i.e., resting-state functional connectivity, rsFC) is associated with upper extremity control in persons approximately one month poststroke. This study examined rsFC within a somatomotor network in persons greater than six months poststroke (n=19). The respective associations between rsFC and multiple parameters representing the continuum of upper extremity behavioral recovery were evaluated. The variance in connectivity explained by these parameters also was determined. Three patterns of somatomotor network connectivity were quantified using fMRI. Volitional activation, control, and real-world use of the affected upper extremity were measured with the Motricity Index, Action Research Arm Test, and accelerometer activity counts, respectively. Results indicated that rsFC between interhemispheric, homotopic regions was stronger than intrahemispheric rsFC in the contralesional and ipsilesional hemispheres. Homotopic connectivity was significantly associated with both upper extremity control (r = .53, p = .02) and real-world use (r = .54, p = .02); however, there was no association with volitional activation of upper extremity muscle groups (r = .23, p =.34). A regression model indicated that the combination of these behavioral parameters accounted for 40% of the variance in rsFC (p = .05). The results reported here are consistent with previous findings, indicating that interhemispheric rsFC strength and its association with upper extremity control are similar in persons with acute and chronic stroke. The association between rsFC and real-world use of the affected upper extremity indicates that connectivity in the resting state provides insight into the activation history of the somatomotor network. Though the continuum of upper extremity behavioral recovery explained 40% of the variance in rsFC, there appear to be other sources influencing somatomotor network connectivity in post-stroke hemiparesis.

Disclosures: M. Urbin: None. **X. Hong:** None. **C. Lang:** None. **A. Carter:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

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Program#/Poster#: 249.10/Z5

Topic: C.22.Stroke Recovery

Support: NSFC 30900709

Title: Focal cerebral ischemia induced motor cortex ascorbate increase is attenuated by pre-ischemia treadmill running

Authors: *K. LIU, Y. WANG, H. WANG, J. LI;
Capital Univ. of Physical Educ. and Sports, Beijing, China

Abstract: Ascorbate plays a key role in brain as an important antioxidant and neuromodulator. Previous studies have demonstrated that ascorbate, as a significant biomarker in cerebral ischemia pathological processes, is influenced by pre-ischemia physical activity. However, the dynamic change of ascorbate in motor cortex in focal cerebral ischemia and its modification with pre-ischemia treadmill running has not been explored. This study aims to investigate the motor cortex ascorbate change in the process of focal cerebral ischemia modified by pre-ischemia running. Adult male Sprague-Dawley rats were randomly assigned into 4 groups. (1) Running and ischemia (RI) group was treated with treadmill running for 2 weeks before undertake a focal middle cerebral artery occlusion (FMCAO). (2) Running and sham operation (RS) group was treated with the same treadmill running for 2 weeks before sham operation. (3) No-running and ischemia (NI) group was treated with FMCAO without pre-ischemia running. (4) No-running and sham operation (NS) group was treated with sham operation without the running. Motor cortex ascorbate level was monitored continuously in 30 min before and in 60 min after the ischemia or sham operation with in vivo microdialysis coupled with on-line electrochemical detection. A passive avoidance test was used to evaluate learning performance 24 hours after ischemia and the next day. The results demonstrate that the motor cortex ascorbate level was increased by FMCAO in 60 min after ischemia. This increased motor cortex ascorbate level was attenuated by 2 weeks pre-ischemia treadmill running. The behavioral results showed a better cognitive function in RI group compared with NI group. These results suggested that pre-ischemia running can modulate ascorbate change induced by FMCAO and is possibly benefit to neural protection.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.11/Z6

Topic: C.22.Stroke Recovery

Title: Gait training with full weight bearing Gait-Assistance Robot for subacute stroke patients with severe gait disturbance: A prospective, randomized, open, blinded-endpoint trial

Authors: OCHI, F. WADA, S. SAEKI, K. HACHISUKA;
Dept. of Rehabil. Med., Univ. of Envrn. and Occup. Hlth., Fukuoka, Japan

Abstract: Objective: To examine whether gait training with a full weight-bearing Gait-Assistance Robot (GAR) improves the gait disturbance in subacute stroke patients more than overground conventional gait training.
Design: Prospective, randomized, open, blinded-endpoint trial
Subjects: Based on the inclusion criteria, 26 non-ambulatory subacute hemiplegic patients were selected from 274 stroke inpatients, and were randomly assigned to the robot-assisted gait training with a GAR (RGT) group and an overground conventional gait training by a physical therapist (OGT) group.
Intervention: The RGT and OGT groups underwent 40 minutes of standard physical therapy and 60 minutes of standard occupational therapy five days a week for four weeks, and 20 minutes of RGT and OGT were added to the two groups, respectively.
Main outcome measures: The severity of hemiplegia was evaluated by determining the Brunnstrom stage, the activities of daily living, the motor score of the Functional Independence Measure (FIM®), gait disturbance, the Functional Ambulation Classification (FAC), the extensor muscle strength of the lower extremities, the StrenthErgo240®; gait speed and by performing the 10 meter Walking Test (10MWT). These outcomes were measured before and four weeks after treatment.
Results: The FAC and extensor muscle strength of the unaffected lower extremities in the RGT group had significantly improved four weeks after treatment (P=0.030 and P=0.007). The gait speed in the RGT group measured with the 10MWT tended to be faster than that in the OGT group after four weeks of treatment (P=0.051).
Conclusions: RGT plus physiotherapy for subacute stroke patients led to a significant improvement in the gait and extensor muscle strength of the unaffected lower extremities in comparison to physiotherapy alone.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Program#/Poster#: 249.12/Z7

Topic: C.22.Stroke Recovery

Support: IRME

ANR-12-JSV4-0007-01

Title: Evidence for ipsilateral corticospinal control in hemiparetic upper limb after cortical lesions as demonstrated by transcranial direct current stimulation

Authors: *W. KLOMJAI[1,2], A. LACKMY-VALLÉE[1], N. ROCHE[3,4], B. BUSSEL[4], P. PRADAT-DIEHL[1,5], R. KATZ[1,5];
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Abstract: Transcranial direct current stimulation (tDCS) has emerged as a method for exploring cortex excitability, and has also begun to be used as a tool to enhance recovery from hemiplegia. Previous studies in healthy subjects have shown that anodal tDCS over the hand motor cortex induces a net increase of reciprocal inhibition between wrist muscles in the contralateral limb while a slight increase of reciprocal inhibition is observed in the ipsilateral limb. It is unknown whether ipsilateral motor activity from the unaffected cerebral hemisphere could be employed after semi-brain damage in patients with paraplegia. To investigate this possibility, reciprocal inhibition of the H-reflex in the forearm flexor muscles was examined in hemiplegic patients at rest. Our results from a small number of patients with unilateral motor deficit showed an increase of reciprocal inhibition in the affected hand during a single application of 20 mins 1.75 mA anodal tDCS over the non-lesioned hemisphere. This increase was greater, and appeared earlier after tDCS onset than in healthy subjects. If the results are confirmed in a subsequent larger study currently underway, this would suggest that ipsilateral motor activity from the unaffected hemisphere provides prominent descending control acting on spinal interneurons, mediating reciprocal inhibition in the hemiparetic limb. As we also found that the level of reciprocal inhibition between wrist muscles at rest was decreased in patients with paraplegia, anodal tDCS could be further developed as a tool for rehabilitation, enhancing reciprocal inhibition between wrist muscles in these patients.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.13/Z8

Topic: C.22.Stroke Recovery

Support: UCB Pharma, unrestricted scientific grant

Title: Effects of GHB and baclofen on sleep and motor function in healthy rats and rats with focal cerebral ischemia

Authors: *A. HODOR, S. PALCHYKOVA, B. GAO, C. L. BASSETTI;
Dept. of Neurol., Univ. Hospital, Inselspital, Bern, Bern, Switzerland

Abstract: Objectives: Ischemic stroke remains one of the leading causes of death worldwide, but no effective treatment is available for the patients. Promotion of neuroplasticity during stroke recovery may represent an alternative therapeutic strategy. Several studies have suggested that neuronal plasticity can be facilitated by sleep. We investigated 1) changes in sleep induced by gamma-hydroxybutyrate (GHB) and baclofen (Bac) in healthy rats, 2) stroke outcome following delayed repeated treatment with Bac in ischemic (isch) rats.
Methods: 1) Rats were implanted with EEG/EMG electrodes. Two doses of GHB and Bac were administered 1h after light onset/offset. 2) Rats were treated with Bac or saline 24h after middle cerebral artery occlusion (MCAo) and then twice daily for 10 consecutive days. Isch/Bac, isch/saline and sham/Bac groups were designed. EEG was recorded during 24-h baseline preceding MCAo and on days 2, 6 and 11 following surgery. Functional recovery was evaluated by single pellet reaching (SPR).
Results: 1) GHB and Bac induced atypical behavior and altered EEG pattern. Vigilance states were evaluated after the end of the drug effect. Bac enhanced NREM sleep in the light and dark phase and REM sleep in the dark phase ($p<0.05$). Moreover, in the light phase Bac increased the duration of NREM sleep episodes, but reduced their frequency, while during the dark only episode frequency was increased ($p<0.05$). Sleep fragmentation was reduced after Bac administration in the dark phase ($p<0.001$). GHB had no major effect on vigilance states. 2) Repeated Bac treatment after MCAo induced changes in vigilance states and motor function. NREM sleep amount was increased significantly during the dark phase in isch/Bac compared to the isch/saline group ($p<0.05$; days 2, 6 and 11). SPR performance dropped to 0 immediately after MCAo in both ischemic groups and recovered slowly thereafter. No significant difference in reaching was found between isch/Bac and sham/Bac groups 33 days after MCAo. In contrast, isch/saline rats never attained the level of sham group and performed significantly worse than isch/Bac rats ($p=0.01$, Tukey-Kramer). Bac had no effect on the size of the brain damage.
Conclusion: Our data showed that 1) GHB and Bac induced sub-anesthetic state distinct from physiological sleep. In contrast to GHB, Bac increased sleep after the end of the drug effect. Time of day determined magnitude of the Bac effect. 2) Delayed repeated Bac treatment might benefit motor function recovery after stroke. This beneficial effect of Bac might be mediated by sleep.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: C.22.Stroke Recovery

Support: NIH 1K01HD069504

Title: Diffusion Tensor Imaging exhibits more proximate and reliable Transcranial Magnetic Stimulation measures compared to fMRI in stroke

Authors: *D. A. CUNNINGHAM[1], A. MACHADO[2], V. RAJAGOPALAN[4], M. LOWE[3], S. JONES[3], E. BEALL[3], K. SAKAIE[3], E. PLOW[1];
[1]Biomed. Engin., [2]Ctr. for Neurolog. Restoration, [3]Diagnos. Radiology, Cleveland Clin., Cleveland, OH; [4]Human Performance and Engin., Kessler Fndn., West Orange, OH

Abstract: In stroke, functional MRI (fMRI) serves as a poor guide to direct Transcranial Magnetic Stimulation (TMS). Localization with fMRI is variable since its hemodynamic contrast is contorted in and around infarcted tissue. Further, fMRI reflects activity in the grey matter, whereas TMS examines conduction via white matter, such as corticospinal tracts (CST). To develop a better guide for TMS in stroke, we examined whether imaging CST terminations in cortices using diffusion tensor imagining (DTI) is more reliable and proximate to sites of TMS than fMRI. Four patients with stroke and four aged-matched healthy controls underwent fMRI during hand movement, and DTI. Stereotactic TMS was delivered to motor cortical sites in a 7 X 5 grid in patients' affected hemispheres while we recorded 5 motor evoked potentials (MEPs) in the paretic first dorsal interosseous (FDI) muscle. We compared the distances between and reliability of MEPs at the following sites: site of highest fMRI activation, cortical sites with best myelin (transverse diffusion) and overall (fractional anisotropy) integrity of CST terminations, and optimal site of TMS (weighted center of gravity of MEPs). Overall, MEPs were reliable when TMS was applied to sites with best integrity of CST terminations; however, when TMS was applied to site of maximum fMRI activation, MEPs were absent in 50% patients and 25% controls. At sites of best myelin integrity of CST, trial-to-trial reliability of MEPs (measured with coefficient of variation) trended towards being better than that at site of maximum fMRI activation (55.58 ± 44.74% vs. 70.32 ± 13.63%) ($p = .07$). In the affected hemisphere, cortical site with best myelin integrity and overall integrity of CST terminations were closer to the optimal TMS site than site of maximum fMRI activation (4.1mm±5.0mm and 5.7mm±6.7mm vs.

14.9mm±3.9mm, respectively) ($p < .05$). Preliminary results of this ongoing work suggest that imaging corticospinal tracts may be reliable and accurate in localizing TMS in stroke. Future studies can explore whether DTI-guidance maximizes outcomes of therapeutic TMS in stroke rehabilitation. Exploring reliable navigation for TMS will allow us to customize therapy to the patient's own neural substrates.

Disclosures: D.A. Cunningham: None. **A. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IntElect Medical, ATI, Cardionomics, Boston Scientific. Consultant/Advisory Board; IntElect Medical, Monteris, Boston Scientific. **V. Rajagopalan:** None. **M. Lowe:** None. **S. Jones:** None. **E. Beall:** None. **K. Sakaie:** None. **E. Plow:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Topic: C.22.Stroke Recovery

Support: Nexstim, Inc

Olson Family Foundation

Faulk Foundation

Title: Improving hand and arm therapy outcomes with rTMS is related to paretic limb motor evoked potentials

Authors: *L. M. ROGERS[1,2], H. R. ROTH[1], R. S. TAPPAN[1], M. B. HARVEY[1], J. W. STINEAR[3], R. L. HARVEY[1,2];
[1]Neuralplasticity Lab., Rehabil. Inst. of Chicago, CHICAGO, IL; [2]Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [3]Univ. of Auckland, Auckland, New Zealand

Abstract: Background: Following stroke, the injured hemisphere has reduced ability to drive movement on the opposite side of the body, impeding function. In addition, the non-lesioned hemisphere (NLH) sends inhibitory signals to the lesioned hemisphere, further reducing its drive to the weak arm and hand. Recent research suggests that the extent of damage to functional and structural integrity of corticospinal pathways originating from the lesioned hemisphere can predict rehabilitation potential. However it is not known whether rehabilitation potential can be improved with enhanced therapy. One enhancement strategy is to reduce the inhibitory signals

from the NLH with low frequency repeated transcranial magnetic stimulation (1hz rTMS) prior to task-oriented occupational therapy (OT). The present study examines whether the functional and structural integrity of corticospinal projections from the lesioned hemisphere are related to therapeutic gains from neuro-navigated 1hz rTMS targeted to the NLH combined with task-oriented OT.
Subjects and Methods: N=30, 3-9 months post-stroke, with incomplete recovery (Chedoke score ≤ 6), randomly assigned to sham, or active 1hz rTMS. At baseline, patients completed TMS assessments of functional integrity (MEP+ or MEP-), anatomical MRI and DTI, and assessments of behavior. Patients then completed 3 therapy visits per week for 6 weeks that included: 20min of pre-functional OT, neuro-navigated 1hz rTMS therapy, and 60min upper-limb task-oriented OT. Once per week TMS recruitment curves were taken from a non-paretic wrist extensor (EDC) both before and after rTMS. Subjects returned for 1 week, 1 month, and 6 month follow-up visits.
Results and Conclusions: Patients receiving active rTMS achieved greater behavioral improvements on the Upper Extremity Fugl Meyer by 6 months post therapy than those receiving therapy alone. Patients with compromised ipsilesional corticospinal tract integrity (MEP-) who received active rTMS achieved significantly greater behavioral gains at all time points following therapy compared to MEP- patients who received therapy alone (sham). For MEP- patients who received rTMS, the degree of behavioral improvement following therapy was significantly correlated with the degree of rTMS induced downregulation in the NLH. These findings suggest that 1hz rTMS targeted to the NLH can be particularly effective in improving the post-stroke rehabilitation potential for individuals with compromised functional integrity of ipsilesional corticospinal projections to the hand and arm.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.16/Z11

Topic: C.22.Stroke Recovery

Support: National Research Foundation of Korea grant 2011-0025938

Title: Differential effects of rtms on genes concerning neural plasticity in young and old rats

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Abstract: Background: Repetitive transcranial magnetic stimulation (rTMS) has been widely used for neuromodulation, however, few mechanisms were well-established on its cumulative effects on neural plasticity. Furthermore, young rats have been used in animal experiments, while rTMS is more commonly applied to the elderly people clinically.
Objectives: To evaluate the differential cumulative effects of rTMS on mRNAs and proteins correlated with the neural plasticity in brain, and to compare these changes between young and old rats.
Methods:
(Experiment 1: selection of the optimal timing of sacrifice after rTMS) To know when is the appropriate time to obtain tissues following rTMS, 8-week-old SD rats were divided into 4 groups (N=20); sacrificed immediate after, 15, 30, and 60 minutes after cessations of rTMS (150% of the motor threshold (MT)) with either high- or low-frequencies. The mRNA of immediate early genes (IEGs) including c-fos, zif268, egr-2 were investigated. (Experiment 2) Unilateral rTMS with low- (1Hz, n=15), high- (20Hz, n=15) frequency, or sham (n=15) stimulations were applied to 8-week (N=45) and 43-week (N=45) old SD rats. Each were divided into 3 subgroups as the number of stimulation sessions; 1-, 5-, and 10-day (n=5 for each subgroup). The mRNAs concerning IEGs (c-fos, zif268), angiogenesis (vegf), neurotrophin (bdnf), long term potentiation and depression (mglur1 and gabar) were performed. Western blots were performed for Akt, PKC, and their phosphorypated forms which are protein kinases related to neurogenesis.
Results: (Experiment 1) In the stimulated hemisphere, zif268 was increased 30 and 60 minutes after high frequency rTMS, while c-fos level was depressed in the contralateral side. Thirty minutes, in which maximal level of IEGs was shown, were selected as a timing of sacrifice in the following experiment. (Experiment 2) Both low- and high-frequency rTMS exerted cumulative effects only in old rats on bdnf ($p=0.024$ and 0.005 respectively), and mglur1 ($p=0.004$ and 0.007 respectively). Low-frequency stimulation had cumulative effects on vegf ($p=0.004$). PKC expression was higher in a 5-day than in 10-day stimulation, with low- and high-frequency rTMS in old rats ($p=0.026$ and $p<0.001$ respectively).
Conclusions: Evidence on cumulative effects of rTMS was shown only in old rats, which correspond to elderly persons, who may be more clinically relevant subjects of rTMS. Furthermore, 5 days of stimulation consistently increased mRNA and protein expression concerning neural plasticity. Five days of duration, rather than 10 days, might be appropriate to the experimental models for rTMS using rats.

Disclosures: J. Kim: None. **B. Oh:** None. **T. Han:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.17/Z12

Topic: C.22.Stroke Recovery

Title: Contracture of finger and hand after cerebral stroke was improved in a short duration by grasping a high repulsion grip

Authors: *H. NISHINO[1], M. SHIRAKI[1], M. YAMAUCHI[1], S. URAKAWA[2], K. SAKAKIBARA[4], H. NISIJO[3];
[1]NPO Corp. Healthy Brain, Nagoya, Japan; [2]Dept of Integ Physiol., [3]Dept of Integ Physiol, Toyama Univ., Toyama, Japan; [4]Sun Barden, Nagoya, Japan

Abstract: In aging society, many persons are attacked by cerebral stroke and suffering from its aftereffects. Rehabilitation is a useful treatment but generally it doesn't work so well. During hand contracture, flexion dominant afferents are sent to the spinal cord and brain, and these would disturb the normal activity. A bouncing cushion made from tangled polyethylene fibers has a high repulsion power and is used in training of football players. We found the blood flow (oxy-Hb) in prefrontal cortex was increased during standing and stepping on the cushion. So we hypothesized if this high repulsion power is delivered to contracted hands, sensory afferents to spinal cord and brain are activated and would offer an appropriate environment for motor output. Under these ideas we designed a high repulsion cushion grip to offer a proper stimulus to contracted hands. We introduced the grip to over 150 patients of brain stroke, Parkinson disease, Alzheimer disease etc. Data were got from 100 patients and in all of them the symptoms were improved in some extent within a week or month: opening of hand and fingers, decrease in muscle tonus, enlargement of joint movement, and disappearance of rash. In some patients who had lost verbal communication recovered mild outlooks and finally spoke a few words. EMG activities recorded from forearm and upper arm muscles increased strongly, and those from jaw and perioral muscles increased moderately during grasping the grip. The oxy-Hb measured by NIRS in the frontal pole area, prefrontal cortex and motor area was increased during intermittent grasping the grip. The stronger patients grasp the grip, the stronger repulsion power is produced and flexor muscles are relaxed. Increase in activity of Ib afferent and decease in activity of Ia afferent, α-,γ-motor neurons of flexor muscles would be the base of the relax. Balance in flexor and extensor activity offers an appropriate environment to promote recovery from contracture. Data indicate that proper afferents from finger and hand offer a natural environment for

sensorimotor coordination in the spinal cord and brain and lead to the improvement of contracture.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Topic: C.22.Stroke Recovery

Support: NIH Grant R01 HD061117-05A2/HD/NICHD

Title: Mapping abnormal functional connectivity in brain injury

Authors: *J. S. SIEGEL[1], N. V. METCALF[2], R. P. FUCETOLA[2], L. RAMSEY[2], C. HACKER[2], A. CALLEJAS[2], A. BALDASSARRE[2], A. Z. SNYDER[2], J. S. SHIMONY[3], G. L. SHULMAN[2], M. CORBETTA[2,3,4];
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Abstract: Evidence from stroke and brain injury literature suggests that cognitive deficit can result from dysfunction distal to areas of tissue damage. Structural brain imaging tools fail to detect pathological changes to brain function in the absence of observable structural damage. Here we present a novel methodology for using resting state functional connectivity MRI (fcMRI) to identify dysfunctional cortex. We apply this method to a patient who has suffered a stroke to the anterior thalamus and a subsequent stable diagnosis of Abulia (loss of will to initiate goal-oriented behavior). This patient's brain shows minimal structural damage that does not adequately explain the profound functional deficit observed. The method presented here compares functional connectivity throughout the patient's gray matter to that of 23 healthy age-matched controls and produces a map of areas in the patient's brain from which connectivity is abnormal. Results identify areas or abnormality proximal to the lesion, as well as small number of regions distal to the lesion. One such region is the dorsal anterior cingulate cortex, a region classically associated with Abulia in lesion studies. Additional experimental evidence presented to validate these results includes 1) a high spatial correlation with a second set of data obtained from the same patient three years later, and 2) data from ten putamen stroke subjects and ten healthy controls that establishes positive and negative controls for this method. Results suggest

that clinically significant functional connectivity changes can occur distal to areas of structural damage and that fcMRI may provide a tool for identifying and localizing brain dysfunction at the level of individual subjects.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Topic: C.22.Stroke Recovery

Support: NIH Grant 1R21NS074244

VA RR&D merit award

Title: Imaging brain activation after motor task in the rat using small animal micro PET

Authors: *S.-Y. TSAI[1,2], S.-H. CHENG[3], H.-M. TSAI[3], H. KIM[3], H. ZHANG[3], L. LEONI[3], G. L. KARTJE[1,2], C.-T. CHEN[3], C.-M. KAO[3];
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Abstract: The use of in vivo imaging has become a powerful tool for studying brain function in either healthy or brain damaged rodents. Although small animal positron emission tomography (μPET) and functional MRI imaging studies have provided help in studying the change of sensory function in a spatiotemporal manner, there is no small animal imaging model available to study corresponding motor activation in the brain.
In this study, we examined whether high-sensitivity small animal mPET imaging can detect and quantify regional differences in brain metabolism/activity corresponding to the performance of a specific motor task.
Male Sprague-Dawly rats were first trained in the skilled forelimb reaching task until successfully obtaining sixteen out of twenty pellets. Animals were then anesthetized and an intravenous catheter inserted into the tail vein. Animals were placed in the forelimb testing box until recovery from anesthesia. 18F-deoxyglucose (FDG, 0.6 mCi) was injected during the following conditions: 1) freely obtain testing sugar pellets placed on the box floor for fifteen minutes, or 2) reaching for sugar pellets placed on the platform as trained for fifteen minutes.

Animals were anesthetized again and scanned with micro CT (mCT) and mPET. mPET images were co-registered with mCT images, and area of interest was outlined and analyzed. Image analysis results showed that there was higher FDG uptake in the corresponding sensorimotor cortex and striatum when the rat performed the skilled forelimb reaching task with the preferred limb. On the other hand, there was no noticable difference in FDG uptake in both sensorimotor cortexes and striata when animals obtained sugar pellets from the floor with their mouths or with symmetric limb movements. These results indicate that high sensitivity small animal mPET can be used for the detection of motor activation and may be very useful in longitudinal studies of the change in brain activation during functional improvement after brain lesions.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Topic: C.21.Perinatal Brain Injury

Support: NICHD, NIH RO1HD069562

Hartwell Foundation

Title: Nanoparticle-mediated therapeutic delivery in a rabbit model of cerebral palsy: Mechanism of nanoparticle uptake

Authors: *E. NANCE[1], B. BALAKRISHNAN[1], F. ZHANG[2], M. MISHRA[3], R. KANNAN[3], S. KANNAN[4];
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Abstract: Cerebral palsy is a chronic childhood disorder that currently has no effective cure. Injury to the developing brain that occurs either in utero or soon after birth can result in motor, sensory and cognitive deficits as seen in cerebral palsy, with neuroinflammation playing a key role in the pathophysiology. Nanoparticles such as dendrimers provide opportunities for the targeted delivery of multiple drugs that can mitigate pathways involved in injury and can be delivered selectively to cells that are responsible for neuroinflammation and injury. We have previously demonstrated that a systemic, postnatal therapy with dendrimer-drug nanodevices in rabbit kits with CP, can cross the disrupted BBB and localize in activated microglia and

astrocytes, resulting in an improvement in motor deficits.
Understanding factors affecting uptake will allow for better design and more efficient delivery of dendrimer platforms to diseased cells, further increasing therapeutic efficacy. In this work, we used a combination of in vivo and ex vivo methods to study the effect of dendrimer size (generation 4 (G4), ~4nm in size) and surface charge (-OH terminated neutral vs -NH2 terminated cationic dendrimers) on the ability to cross an impaired BBB, diffuse within the brain parenchyma, and selectively uptake in microglia cells in rabbit kits exposed to endotoxin in utero. In brain slices from endotoxin kits, the neutral G4-OH-Cy5 dendrimer could diffuse through the brain parenchyma, preferentially accumulating in microglia, whereas the cationic G4-NH2-FITC dendrimers did not diffuse in slices. Following intracranial administration into an endotoxin day 1 kit, G4-OH dendrimer was found distributed in the parenchyma at 4 hrs and 24 hrs, and localized in microglial cells at 24 hrs. In age-matched healthy controls, minimal uptake in microglia was seen in either white matter or cortical regions; however, dendrimer was spread throughout the parenchyma at 24 hours confirming the diffusive nature of these particles. Following intravenous (IV) administration, G4-OH-Cy5 dendrimer penetrate a disrupted BBB in endotoxin kits and were found within the parenchyma at 4 hours, and within microglial cells at 24 hours. Polystyrene nanoparticles (20nm) did not cross the BBB or selectively uptake into microglia cells. Dendrimer was not found in the brain in age-matched healthy controls following systemic administration. These results suggest that appropriate tailoring of the physicochemical properties of the dendrimer nanodevice to optimize targeting and localization can lead to improvement in drug delivery to the brain.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Topic: C.22.Stroke Recovery

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ICTS at UC Irvine UL1 TR000153

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Title: Cortical connectivity predicts motor impairment and improvement with Telerehabilitation therapy in chronic stroke

Authors: *J. WU, R. SRINIVASAN, N. KATHURIA, L. DODAKIAN, J. SEE, S. C. CRAMER;
Univ. of California, Irvine, Irvine, CA

Abstract: A number of predictors of response to rehabilitation therapy after stroke have been identified. Some of these rely on MRI scanning, however, expense and acquisition demands can limit access to such data. Dense-array electroencephalography (dEEG) may be a useful tool for improving prediction, as this technique is safe, inexpensive, easy to use, and can be employed at the bedside to characterize brain function. In this study, dEEG was used to predict motor impairment and response to therapy in patients with chronic stroke enrolled in a Telerehabilitation study. METHODS. Ten adults (mean age = 53.8±16.9 years) with chronic stroke and mild to moderate hemiparesis (mean Fugl-Meyer (FM) score = 41.4±11.6, normal = 66) underwent 3 minutes of resting-state dEEG recording prior to 4 weeks of Telerehabilitation therapy. Analyses were performed with custom MATLAB code. Continuous raw dEEG were filtered, segmented, and cleaned of artifacts with visual inspection and Infomax-Independent Component Analyses. Mean coherence with an ipsilesional M1 (iM1) seed region was derived for the high beta (20-30 Hz) frequency band, which is known to capture motor network interactions. Partial least squares (PLS) regression was used to predict baseline FM score and change in FM score using resting-state mean beta coherence with iM1. A leave one out and validate procedure was used to determine predictive strength of the model. RESULTS. With therapy, the group demonstrated significant improvement on the FM scale (mean FM change = 4.7±2.8, p=0.0005, 2 tailed paired t-test). The PLS model using beta coherence with iM1 to predict baseline FM score revealed ipsilesional parietal (iPr) and premotor (iPM) regions, with r^2=0.92. The PLS model using beta coherence with iM1 to predict improvement in FM score revealed iPM, iPr, midline supplementary motor area, and contralateral M1 regions, with r^2=0.61. CONCLUSIONS. Resting-state measures of cortical connectivity were able to predict both baseline impairment and response to 4 weeks of therapy in individuals with chronic stroke with a very strong degree of accuracy.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Alberta innovates health solutions

Heart and stroke foundation of Alberta

University hospital foundation

Canada foundation for innovation

NSERC CRSNG

Faculty of medicine & dentistry, University of Alberta

Title: Changes in collateral blood flow and neuroprotective efficacy of transient aortic occlusion during acute ischemic stroke

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Abstract: Stroke is the rapid loss of brain function, due to blockage or haemorrhage of a cerebral blood vessel. The occlusion of a cerebral blood vessel results in oxygen and nutrient deprivation in the tissue leading to an ischemic stroke. The region immediately downstream of the occlusion is the ischemic core, which suffers fast and irreversible tissue damage due to blood flow reduction to below 20% of baseline flow. The region surrounding the ischemic core has partially preserved perfusion of 30-40% baseline blood flow and is called an ischemic penumbra. This partial blood flow to the ischemic territories is maintained via collaterals that provide an alternate passage for blood flow to reach the ischemic tissue when the principal conduits fail. Augmenting blood flow through the cerebral collaterals might further protect the tissue and reduce the damage caused by an ischemic insult. Here, we evaluate the efficacy of a novel form of collateral therapy, transient aortic occlusion (TAO), to increase collateral blood flow during stroke in two models of middle cerebral artery occlusion (MCAo) in rodents. The blood flow changes were monitored by laser speckle contrast imaging (LSCI), which provides a high resolution maps of cortical blood flow from which relative changes in blood flow velocity and diameter determined. Analysis of LSCI maps acquired after thromboembolic MCAo (using a clot volume designed to selectively occlude the MCA) identified significant increases in blood flow velocity and vessel diameter in MCA segments downstream of anastomoses between the middle and anterior cerebral arteries (MCA, ACA) during TAO treatment. Notably, enhanced collateral blood flow persisted even after the TAO treatment in thromboembolic MCAo rats. To evaluate blood flow augmentation in a model of stroke highly resistant to recanalization therapies, we next used a filament occlusion model to induce a stable, proximal occlusion of the MCA/internal carotid (mimicking a tandem ACA/MCA occlusion). In this model, LSCI did not identify

persistent increases in blood flow velocity or vessel diameter in MCA segments downstream of MCA-ACA anastomoses after TAO treatment. These data support preliminary clinical findings suggesting that TAO is most effective in strokes of moderate size, and highlights the importance of patient selection in evaluation of acute stroke therapies.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Topic: C.22.Stroke Recovery

Support: UM BKP001-2012A

Title: Modulatory effects of three different non-invasive brain stimulation techniques

Authors: *H.-T. GOH[1], L. ABDUL LATIF[2], H.-Y. CHAN[2];
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Abstract: High frequency repetitive transcranial magnetic stimulation (HF-rTMS) has been found to be an effective tool to up-regulate corticospinal excitability of primary motor cortex (M1). The technique is expensive and maybe unsafe because of its potential of triggering seizure. Compared to HF-rTMS, anodal transcranial direct current stimulation (A-tDCS) is cheaper and safer to up-regulate corticospinal excitability; however, it is less focal and powerful. Previous study has shown that inhibitory low frequency rTMS (LF-rTMS) applied to dorsal lateral prefrontal cortex (DLPFC) increased premotor activity (Gangitano et al. 2008). LF-rTMS is safer than HF-rTMS while providing the same level of preciseness. To date, there is no study directly comparing these three different non-invasive brain stimulation techniques on their effectiveness on up-regulating M1 excitability. The purpose of this study was to compare the effectiveness of HF-rTMS, LF-rTMS and A-tDCS on corticospinal excitability of M1.
Eight healthy participants (mean age = 28 years; 4 males) participated in the study. Each participant went through 3 different stimulation sessions, namely HF-rTMS, LF-rTMS and A-tDCS. Each session was at least 1 week apart from the other and the order was counterbalanced among participants. For HF-rTMS, 5Hz rTMS at 90% of RMT was structured into 24 10s trains with an inter-train interval of 30s (total pulse = 1200) and applied to the M1 of the non-dominant hemisphere. For LF-rTMS, 1Hz rTMS (90% RMT) was continuously applied to the DLPFC for 20 minutes (total pulse = 1200). We applied 20 minute 1mA tDCS to M1 via anodal electrode

during A-tDCS session. M1 corticospinal excitability was determined by motor evoked potential (MEP) using single pulse TMS. MEP was measured before stimulation, immediately after, 15 minutes, 30 minutes and 60 minutes after stimulation. Behavioral changes were assessed. Significant increase in MEP measured at M1 was found after stimulation ($p = .00$). The increase lasted up to 60 minutes post-stimulation. There was no significant effect of stimulation type ($p = .31$) or interaction between stimulation type and time of measurement ($p = .16$). Participants' performance on Trail Making Test improved significantly after stimulation ($p = .00$). Both A-tDCS and LF-rTMS led to significant improvement on Trail Making Test while HF-rTMS did not.
These findings suggest that all three stimulation techniques may be equally effective in up-regulating M1 excitability while the rTMS seemed to have a longer lasting effect than tDCS. LF-rTMS applied to DLPFC could be used as an alternative way to increase M1 excitability other than HF-rTMS.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Program#/Poster#: 249.24/AA1

Topic: C.22.Stroke Recovery

Support: NIH grant NS038684

Title: Transfusion of carbon monoxide-bound pegylated hemoglobin during reperfusion from transient focal cerebral ischemia reduces infarct volume

Authors: *X. LIU, H. KWANSA, E. KULIKOWICZ, J. ARMSTRONG, D. SPICER, R. C. KOEHLER;
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Abstract: Early transfusion of carbon monoxide-bound pegylated hemoglobin (PEG-COHb) helps to maintain pial arteries in a dilated state and reduces infarct volume when transfused at 20 min of a 2-h period of middle cerebral artery occlusion (MCAO) (Zhang et al. J Appl Physiol 2012; 113:1709). The CO is quickly released from the Hb, which is converted into an oxygen carrier. Because PEG-COHb was superior to PEG-Hb without CO, the small amount of released CO provides additional protection. Here, we determined if delaying transfusion until after reperfusion also is protective. Male rats weighing 300±10 g were subjected to 2 h of MCAO by the filament technique and 2 days of reperfusion for triphenyltetrazolium chloride-determination

of infarct volume. Three groups of rats (n = 10-12) were transfused with 10 ml/kg of a 4% solution of PEG-COHb over 6 min starting at 20 min, 2 h, or 4 h after onset of MCAO. Infarct volume in cerebral cortex (% of ipsilateral structure) was significantly decreased from 36.1 ± 5.1% (SE) in a normal saline-treated control group to 19.2 ± 7.0% in the group transfused at 20 min, to 14.9 ± 4.6% in the group transfused at 2 h of MCAO (onset of reperfusion), and to 17.4 ± 4.9% in the group transfused at 4 h of MCAO (2 h of reperfusion). Infarct volume in striatum also was significantly decreased from 48.8 ± 5.4% in the control group to 21.3 ± 6.7% in the group transfused at 20 min and to19.1 ± 6.3% in the group transfused at 2 h. However, infarct volume in striatum was no longer significantly different (33.4 ± 7.8%) when PEG-COHb was transfused at 4 h after MCAO, consistent with the rapidly evolving cell death in striatum. These data indicate a significant therapeutic time window for transfusion of small amounts of PEG-COHb after ischemic stroke and that protection may be additive to that associated with early reperfusion.

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Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

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Program#/Poster#: 249.25/AA2

Topic: C.22.Stroke Recovery

Support: Wellcome Trust Funding: WT090911MA

Title: Measuring vascular reactivity with breath-holds after stroke: Implications for fMRI study interpretations

Authors: *F. GERANMAYEH[1], R. J. S. WISE[1], K. MURPHY[2];
[1]Imperial Col. London, London, United Kingdom; [2]Cardiff Univ. Brain Res. Imaging Ctr., Cardiff, United Kingdom

Abstract: Introduction:
Blood oxygenation level dependent (BOLD) contrast fMRI is a widely used technique to map brain function after stroke. Changes over time after stroke in the coupling between blood flow and neural activity may result in problems with interpretation, particularly in the acute phase when neurovascular decoupling may be maximal. This study measured within- and between-subject differences in vascular reactivity in stroke patients and normal participants.
Methods:

38 patients (mean age 61 years, range 26-79, 26 male) with left hemisphere infarct underwent a breath-holding (BH) paradigm similar to that published previously1. Of these, 18 subjects were scanned in the subacute phase post stroke (SPPS) (mean 15 days), 11 of whom had a second scan in the chronic phase post stroke (CPPS). A further 14 patients were scanned in the CPSS. The 25 scans in the CPPS were obtained at a mean of 113 days post stroke. 26 healthy older controls (mean age 57 years, range 37-78, 9 male) were also scanned.
End tidal CO2 (ETCO2) traces were measured during the breath-holds and a vascular reactivity measure was derived as a percentage BOLD signal increase per mmHg rise in ETCO2. Vascular reactivity maps were registered to standard space. The mean reactivity was calculated in the lesion, lesion penumbra, lesion homologue in right hemisphere, and the entire left (LH) and the right hemispheres (RH).
Results:
In both APPS and CPPS, the penumbra displayed higher vascular reactivity measures than the lesion ($P<0.004$). In both APPS and CPPS, the penumbra also had reduced vascular reactivity compared to the lesion homologue ($P=0.008$ APPS, 0.011 CPPS) and the RH ($P=0.01$ APPS, 1x10-6 CPSP). The RH vascular reactivity in APPS and CPPS was no different from the whole brain reactivity in controls.
Of the 11 patients who had data in both the APPS and CPPS, 1 subject was excluded as reactivity in APPS was >2SD away from the mean of all 18 APPS scans. Of the remaining 10, there was a significant increase in vascular reactivity over time in both hemispheres ($P= 0.02$ LH, 0.04 RH).
Conclusion:
Since vascular reactivity is reduced, lack of fMRI activity in the stroke penumbra should be treated with caution early after stroke. The fMRI activity in the unaffected hemisphere is less likely to be contaminated by vascular reactivity alterations at these time points. Inclusion of vascular reactivity response as a voxel-dependent covariate in fMRI studies of lesioned brain may be necessary to account for regional and between subject variability in BOLD response after stroke.
1: Neuroimage. 2011 1;54(1):369-79.

Disclosures: F. Geranmayeh: 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.; Wellcome Trust funding for research training fellowship. **R.J.S. Wise:** 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.; MRC funding for research. **K. Murphy:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.26/AA3

Topic: C.22.Stroke Recovery

Support: NIH Grant NS057255

NIH Grant NS073378

American Heart Association (AHA) Established Investigator Award

AHA Postdoctoral Fellowship (12POST12080252)

Title: Optogenetic stimulation of the striatum promotes functional recovery after focal ischemic stroke in mice

Authors: *M. SONG, O. MOHAMAD, X. GU, S. WEI, L. WEI, S. YU;
Dept. of Anesthesiol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Optogenetics has emerged as an innovative and promising technology in mapping neuronal infrastructure and functional activities at a highly accurate and specific level. It also provides an opportunity to develop new therapies for nervous system disorders. The present investigation tested whether optogenetic technique can be translated into stroke treatment by photo-stimulation of the striatum based on the notion that the striatum region of the brain may support self-repair process after cerebral ischemia. Focal ischemic stroke was induced in male adult Channelrhodopsin-2 (ChR2) transgenic mice, taking the advantage of high expression of the light sensitive cation channel ChR2 in the striatum. Electrophysiological recording on brain slices from these mice showed channel activation upon flashing blue laser light (473 nm). Before stroke, mice were trained 5 times per day for 3 days with a modified adhesive removal test. Mice were then subjected to the ligations of middle cerebral artery branches, targeting the right sensorimotor (barrel) cortex. Four days after stroke, optical fibers were implanted into the striatum and fixed with a cannula on the skull. In control group, stoke mice received optical fiber implantation but without photo-stimulation. In treatment group, daily photo-stimulation pulses were applied at 5 days after stroke and repeated for 8 days. Intensity of the blue laser light was set below the threshold to induce excessive body movement. The adhesive removal test on forepaws was performed 3, 10, 17, 24, and 31 days after stroke. The impaired forepaw sensorimotor function in these two groups progressively recovered over the timeline. Stroke mice treated with photo-stimulation, however, showed significantly better recovery detected 31 days after stroke compared to stroke control. Current experiments are exploring the underlying mechanism of the optogenetic treatment in the post-ischemic brain.

Disclosures: M. Song: None. **O. Mohamad:** None. **X. Gu:** None. **S. Wei:** None. **L. Wei:** None. **S. Yu:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.27/AA4

Topic: C.22.Stroke Recovery

Support: AIF Grant 193639

Title: Voluntary forced use of the impaired forelimb facilitates neurological recovery after focal cerebral ischemia in the rat

Authors: *J. LIVINGSTON-THOMAS, T. A. DOUCETTE, R. A. TASKER;
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Abstract: Constraint induced movement therapy (CIMT), which forces use of the impaired arm following unilateral stroke, promotes functional recovery in the clinic. Modeling CIMT in animals has proven challenging, and has yielded mixed results. Our aim was to develop a refined endothelin-1 (ET-1) model of focal ischemic injury in rats that results in reproducible, well-defined lesions and reliable upper extremity impairments, and to determine if an appetitively motivated form of forced use rehabilitation (REHAB) would accelerate post-ischemic motor recovery. Male Sprague Dawley rats (N = 23) were subjected to focal unilateral stroke via intracerebral microinjections of endothelin-1 (ET-1) to forelimb motor regions, or sham surgery. Three days later, ET-1 animals were then assigned to either daily REHAB or CTRL therapy. REHAB consisted of 30 minutes of voluntary generalized movement (exercise ball) sessions, followed by 30 minutes of voluntary task-specific movement (pellet reaching). A number of behavioural tests of forelimb function were used to determine the effect of REHAB on functional recovery, and histological and immunohistochemical examinations were performed to assess lesion volume and expression of markers of neuroplasticity. We found that voluntary forced use of the impaired forelimb results in accelerated functional recovery in forelimb placing and staircase reaching, without significantly affecting infarct volume. Animals that received REHAB had significantly more migrating neuroblasts in the ipsilesional subventricular zone and perilesional tissue than sham controls. Furthermore, REHAB altered the cellular origin of expressed BDNF, resulting in more expression from non-neuronal, non-astrocytic cells. Our results suggest that improved appetitively-motivated forced use rehabilitation protocols may prove useful in developing new strategies to improve rehabilitation and for mechanistic studies

of functional neuroplasticity.
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Disclosures: J. Livingston-Thomas: None. **T.A. Doucette:** F. Consulting Fees (e.g., advisory boards); Neurodyn, Inc. **R.A. Tasker:** F. Consulting Fees (e.g., advisory boards); Neurodyn, Inc..

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.28/AA5

Topic: D.13. Motor Neurons and Muscle

Support: The Development of Robot-Assisted Motor Rehabilitation of the Upper Limb Using Bio-Signal Interfaces Project of the Korea Institute of Science and Technology (KIST), Korea has supported this work.

Title: A comparison of eeg patterns during active and passive control of haptic devices in stroke patients

Authors: *W. PARK[1], J.-H. KANG[2], G.-H. KWON[1], W. CHANG[4], Y.-H. KIM[4], L. KIM[1], S.-P. KIM[3];
[1]Ctr. for Bionics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]Dept. of Brain and Cognitive Engin., [3]Res. and Business Fndn., Korea Univ., Seoul, Korea, Republic of; [4]Dept. of Physical and Rehabil. Med., Samsung Med. Center, Sungkyunkwan Univ. Sch. of Med., Seoul, Korea, Republic of

Abstract: Effective rehabilitation therapies in the acute stage can improve functional recovery and minimize impairments. Robot-assisted rehabilitation therapies have been developed to overcome the problem of labor-intensive work in the traditional physical therapy. Recently, an approach to combine brain-computer interfaces (BCIs) with the robot-assisted technology has emerged, enabling volitional and asynchronous control of robots by detecting movement intentions via BCIs. A prerequisite to this approach includes understanding how brain activity in stroke patients is modulated during the rehabilitation training with robotic devices. Hence, the present study investigates how brain activity patterns change when the patients volitionally control devices in rehabilitation training. Specifically, we aim to find differences in electroencephalography (EEG) patterns during active and passive control of a haptic device. Eleven chronic stroke patients participated in the study and were asked to grasp or supinate the

handle of a haptic device with active or passive control modes. The haptic device operated regardless of the patient's intention in the passive mode whereas it was completely moved by the patients in the active mode. Each trial was composed of three successive periods, including the task period (2s), the hold period (1s) and the return period (2s). We recorded 64-channel EEG in the patients during the experiment. After processing EEG signals to extract event-related synchronization/desynchronization (ERS/ERD) of the sensorimotor rhythms, we extracted features of temporal ERS/ERD patterns, including a minimum peak, a slope of ERD and the area under curve (AUC), and analyzed the effects of different tasks (active and passive) on these features. In the task period, paired sample t-tests revealed significantly greater minimum peak values and stiffer slopes of ERD during the active mode than during the passive modes ($p < 0.05$). In the hold period, tests revealed significantly greater minimum peak values and larger AUC during the active mode than during the passive mode ($p < 0.05$). These results demonstrated that volitional hand movement in stroke patients' elicited different EEG patterns from those by passive hand movement. Our results may provide a basis for further development of an index of how actively stroke patients are engaged in rehabilitation training.

Disclosures: W. Park: None. **J. Kang:** None. **G. Kwon:** None. **W. Chang:** None. **Y. Kim:** None. **L. Kim:** None. **S. Kim:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.29/AA6

Topic: C.09. Ischemia

Support: AHA Grant 10PRE3050053

NIH Grant NS45676

NIH Grant NS054147

NIH Grant NS34773

Lois Pope Life Fellows Program

Title: Protein kinase C epsilon regulates mitochondrial NAD+/NADH following resveratrol and ischemic preconditioning in cortical cultures

Authors: *K. MORRIS, J. T. NEUMANN, C. H. COHAN, S. V. NARAYANAN, M. A. PEREZ-PINZON;
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Abstract: Following cerebral ischemia, severe reductions in NAD^+/NADH cause energy depletion and neurodegeneration [1]. Preserving mitochondrial pools of nicotinamide phosphoribosyltransferase (Nampt), the major biosynthetic pathway for NAD^+/NADH production, confers cytoprotection when cytoplasmic NAD^+/NADH is depleted[2]. However, the signaling pathways involved in regulating mitochondrial Nampt are currently unknown. Nampt overexpression has been shown to protect the brain against ischemic injury in via and AMP-activated protein kinase (AMPK) pathway[3]. Since both AMPK and protein kinase C epsilon (PKCε) enhance mitochondrial function and ischemic neuroprotection, we hypothesized that these enzymes work together to regulate mitochondrial-localized Nampt and NAD^+/NADH during resveratrol (RPC) or ischemic preconditioning (IPC), a paradigm where a brief ischemic insult protects the brain against a subsequent lethal ischemic insult. Preconditioning was induced by exposing mixed cortical cultures to 1h exposure of oxygen-glucose deprivation (IPC), resveratrol (RPC), ψεRACK (PKCε agonist; εPC), AICAR (AMPK activator; APC), εV1-2 (PKCε antagonist) or Compound C (AMPK inhibitor; CC) and the mitochondria were assessed 48h later. Nampt protein levels were increased in purified mitochondrial fractions following εPC and APC which were blocked with exposure to CC and εV1-2, respectively. IPC and RPC required PKCε, but not AMPK, to mediate increased mitochondria-localized Nampt ($p < .01$, $n = 6$) indicating PKCε was essential for regulating mitochondrial Nampt levels. Assessment of NADH autofluorescence using 2-photon microscopy revealed larger delta changes in NADH fluorescence following cyanide treatment in εPC-treated groups in comparison to controls ($p<.001$, $n=6$), indicating PKCε activity increased the NAD^+/NADH ratio. Biochemical analysis of NAD^+/NADH revealed that εPC increased mitochondrial concentrations of NAD^+, NADH, and the NAD^+/NADH ratio ($p < .01$, $n = 5$) in an AMPK and Nampt-dependent manner. During IPC and RPC, PKCε activity was required for increases NAD^+ concentrations and the NAD^+/NADH ratio. Collectively these results showed that PKCε is a major regulator of mitochondrial pools of Nampt and NAD^+/NADH during IPC and RPC. Furthermore, these findings suggest a novel mechanism by which the PKCε-AMPK pathway provides protection to brain mitochondria against ischemic stress.

References
1. Iwashita et al. (2004). J Pharmacol Exp Ther.
2. Yang et al. (2007). *Cell*
3. Wang et al., (2011). *Ann Neurol.*

Disclosures: K. Morris: None. **J.T. Neumann:** None. **C.H. Cohan:** None. **S.V. Narayanan:** None. **M.A. Perez-Pinzon:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

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Program#/Poster#: 249.30/AA7

Topic: C.09. Ischemia

Support: National Biophotonics and Imaging Platform Ireland (NBIP) funded through the Higher Education Authority (PRTLI Cycle 4)

Title: AMP-activated protein kinase (AMPK)-induced preconditioning in primary neurons requires MCL-1

Authors: *U. ANIL KUMAR[1], P. WEISOVÁ[1,2], H. DÜSSMANN[1], C. G. CONCANNON[1], H. G. KÖNIG[1], J. H. M. PREHN[1];
[1]Dept. of Physiol. and Med. Physics and RCSI Neurosci. Res. Cen, Royal Col. of Surgeons In Ireland, Dublin, Ireland; [2]Max F. Perutz Laboratories, Univ. of Vienna, Vienna, Austria

Abstract: Neuronal preconditioning is a phenomenon where a stressful but not damaging stimulus activates an endogenous adaptive response to reduce the impact of subsequent, more severe stimuli. Activation of the energy stress sensor, AMP-activated protein kinase (AMPK) has been shown to contribute to the protective effects of ischemic, excitotoxic and mitochondrial uncoupling-induced preconditioning in neurons, however the molecular factors contributing to AMPK-induced preconditioning have been less well characterised. Here we investigated the role of AMPK signaling pathways during 5-aminoimidazole-4-carboxamide riboside (AICAR) preconditioning against NMDA-mediated excitotoxicity in primary mouse cortical neurons. Activation of AMPK with low concentrations of AICAR (0.1 mM for 2 h) induced a sustainable increase in the phosphorylation state of AMPK, and protected neurons against NMDA-induced excitotoxicity. Conversely, AMPK gene silencing abolished protection against NMDA-induced excitotoxicity. By characterizing potential bioenergetic, transcriptional and post-translational effects of transient AMPK activation, we observed a marked increase in mRNA expression and protein levels of both MCL-1^{OM} (40 and 38 kDa) and MCL-1^{Matrix} (36 kDa) form of the anti-apoptotic BCL-2 family protein MCL-1 in AICAR-preconditioned neurons. Interestingly, overexpression of MCL-1 protected neurons against NMDA-induced excitotoxicity but did not show any further significant protection in AICAR-preconditioned neurons. Conversely, MCL-1 gene silencing was sufficient to induce cell death, and abolished the effect of AICAR preconditioning. Gene silencing or inhibition of AMPK attenuated the increase in mRNA expression and protein levels of MCL-1. To identify the mechanism through which MCL-1 confers neuroprotection during excitotoxicity, we monitored intracellular Ca^{2+} levels during NMDA excitation, and noted that MCL-1 overexpressing neurons exhibited markedly reduced

Ca^{2+} elevations. Furthermore, MCL-1 gene silencing increased the NMDA-mediated Ca^{2+} elevations in AICAR preconditioned neurons. Therefore this study identifies MCL-1 as a key effector of AMPK-induced preconditioning in neurons.

Disclosures: U. Anil Kumar: None. **P. Weisová:** None. **H. Düssmann:** None. **C.G. Concannon:** None. **H.G. König:** None. **J.H.M. Prehn:** None.

Poster

249. **Stroke Recovery: Integrated Responses and Treatment**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 249.31/AA8

Topic: C.09. Ischemia

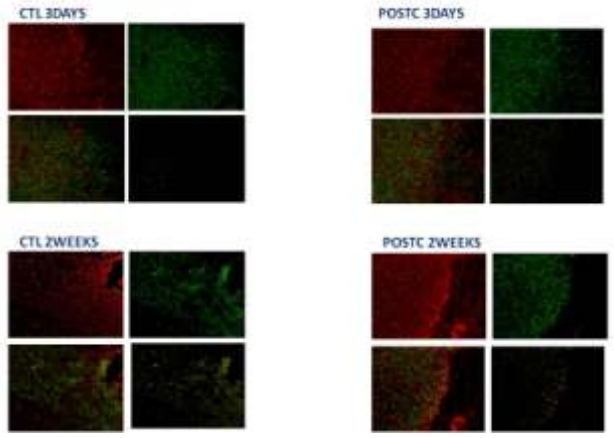
Support: NIH Grant R37NS37074

Title: Effects of ischemic postconditioning on the progression of focal cerebral ischemia

Authors: *E. ESPOSITO[1,2], K. HAYAKAWA[2], K. VAN LEYEN[2], S. POLI[1], U. ZIEMANN[1], E. LO[2];
[1]Hertie Inst. For Clin. Brain Res., Tübingen, Germany; [2]Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Ischemic postconditioning is an endogenous neuroprotective mechanism that has recently been shown to be broadly effective after ischemic stroke. Postconditioning is thought to recruit natural adaptive responses that the brain and other organs utilize to protect themselves from various insults (Pignataro et al., 2008, Pignataro et al., 2009, Zhao, 2007, Zhao, 2009, Zhao et al., 2006 and Zhao et al., 2012, Pignataro, et al., 2013). To be clinically relevant, long-term safety and efficacy must be further explored. Here, we assessed the effects of postconditioning after 100 min of focal cerebral ischemia induced by intraluminal middle cerebral artery occlusion (MCAO) in adult male Sprague-Dawley rats. Reperfusion was established for 10 minutes after which postconditioning was performed by re-occluding the MCA for another 10 minutes (Pignataro, et al., 2008).
The rats were randomized into a control vs. postconditioning group and analyzed 3 days or 2 weeks post reperfusion. Infarct volumes and behavioral outcomes were blindly quantified (Fujiwara, et al., 2011, Singhal AB, et al., 2002). Immunohistochemistry was used to examine the effects of postconditioning on glial activation; GFAP, a marker of activated astrocytes and BDNF, a neurotrophic factor, were analyzed (Hayakawa, et al., 2010).
After 3 days and 2 weeks of reperfusion the infarct volume was markedly reduced in animals subjected to postconditioning. They also had better outcomes in forelimb placement test and in

body-swing test. Reactive astrocytes co-expressing GFAP and BDNF increased in peri-infarct cortex at 3 days after postconditioning compared to controls while there was no longer a difference after 2 weeks.
These results suggest that neuroprotective effects of postconditioning may persist for up to 2 weeks post-stroke in a model of transient focal ischemia and that astrocytes may be involved in this neuroprotection at different stages after ischemia.



Disclosures: E. Esposito: None. **K. Hayakawa:** None. **K. van Leyen:** None. **S. Poli:** None. **U. Ziemann:** None. **E. Lo:** None.

Poster

250. **Ischemia: Animal Models**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 250.01/AA9

Topic: C.09. Ischemia

Support: Grant from Fondazione Cassa di Risparmio di Pisa

Title: Behavioral and neuroanatomical changes following focal motor cortex ischemia in the mouse

Authors: C. ALIA[1], C. SPALLETTI[2], S. LAI[3], M. MAINARDI[4], A. PANARESE[3], S. MICERA[5,3], *M. CALEO[4];
[1]Scuola Normale Superiore, Pisa, Italy; [2]Inst. of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy; [3]The BioRobotics Institute, Scuola Superiore Sant'Anna, Pisa, Italy; [4]Neurosci. Institute, C.N.R., Pisa, Italy; [5]Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

Abstract: Stroke is one of the leading causes of long-term motor disabilities. There is therefore a pressing need for a more complete understanding of the mechanisms of post-infarct brain plasticity that mediate both spontaneous and rehabilitation-induced motor recovery. Such knowledge can be gained in appropriate animal models, and in this context, the mouse allows carrying out detailed mechanistic investigations.
Here, we have assessed behavioral and neuroanatomical changes following focal photothrombotic stroke in the caudal forelimb area of the mouse. In particular, we have evaluated the time course of the consequent motor deficits using classical behavioral tests such as the Gridwalk Test, the Schallert Cylinder Test and the Swim Test. We have also performed anatomical investigations using immunostaining for neuroplasticity markers at different time-points after the ischemic lesion (7, 21 and 30 days).
Behavioral outcomes indicated a reliable motor deficit that was selective for the contralesional forelimb and persisted for at least 30 days after phototrombotic stroke. Neuroanatomical analysis in the peri-infarct cortex showed a decrease in the density of perineuronal nets and parvalbumin-positive interneurons. We are currently examining other neuroplasticity markers such as the ratio of excitatory and inhibitory terminals, and the expression of the neurotrophin BDNF. We are also using intracortical microstimulation to map rearrangements in the motor cortex following stroke. These experiments provide information on the spontaneous evolution of behavioral, electrophysiological and neuroanatomical measures following focal cortical infarction in the mouse. The data will be important for understanding the correlates of rehabilitation-induced motor recovery. In particular, the same measures will be extracted from mice subjected to post-stroke rehabilitation with a robotic device that we have recently described (Spalletti and Lai et al., Neurorehabil Neural Repair, 2013).

Disclosures: C. Alia: None. **C. Spalletti:** None. **S. Lai:** None. **M. Mainardi:** None. **A. Panarese:** None. **S. Micera:** None. **M. Caleo:** None.

Poster

250. **Ischemia: Animal Models**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 250.02/AA10

Topic: C.09. Ischemia

Support: DST Extramural Grant, SR/SO/HS-0001/2010

CSIR Junior Research Fellowship, 09/141(0176)/2011-EMR-1

Title: Establishment of pterygopalatine artery (PPA) ligation murine model of retinal ischemia to test efficacy of stem cells

Authors: *G. MINHAS, S. PRABHAKAR, A. ANAND;
Dept. of Neurology, Post Grad. Inst. of Med. Educ. & Res., Chandigarh, India

Abstract: Current treatments available for retinal ischemia are not sufficient to restore the visual functions. The replenishment of neurons and retinal cells by transplanted stem cells has been given increasing attention to treat retinal diseases (Singh et al., Stem Cells & Dev 2011; Tomita et al., Stem Cells 2002). Retinal ischemia is a major cause of blindness and is associated with a wide range of clinical disorders like ischemic optic neuropathies, obstructive retinopathies, carotid occlusive disorders, diabetic retinopathy, glaucoma and stroke (Osborne et al., Prog Retin Eye Res 2004). Different animal models of retinal ischemia have been generated to study its pathophysiology and to test potential therapeutics. The models that have been established to study retinal ischemia are high intraocular pressure, optic nerve ligation and vascular models. Each of these models has its own disadvantages. The recent development is the pterygopalatine artery (PPA) ligation model to study transient retinal ischemia (Lelong et al., Stroke 2007; Ogishima et al., IOVS 2011) which has an advantage over the middle cerebral artery occlusion (MCAO) model (Block et al., Neuroscience Lett 1997; Steele et al., Stroke 2008), has a problem of poor reproducibility and high mortality rate leading to problem in evaluating retinal ischemia. The intraluminal suture MCAO model in C57BL/6J mice with 1 hour of occlusion and 23 hours of reperfusion showed an approximate 42% mortality within 24 hours of occlusion. In this study the PPA ligation model was established and characterised. Age-matched and sex-matched C57BL/6J mice were subjected to PPA ligation. The external carotid artery and the pterygopalatine artery were ligated for 3.5 hours which reduces the ocular blood flow. After 5 days of reperfusion, the mice were sacrificed and the eyes were enucleated. The retinal damage was assessed against controls using histological, molecular and immunohistochemical techniques. The glial fibrillary acidic protein (GFAP) expression was evaluated in retina and the expression was found to be up-regulated at both the protein and mRNA level. The immunohistochemical analysis demonstrated an increase in GFAP expression in retinal cryosections as compared to the control sections. Similarly an increase in GFAP mRNA levels was manifested in real-time PCR from the cDNA synthesised from whole retina homogenate. Thus, this murine model of transient retinal ischemia will be used to check the migration and differentiation efficacy of lineage negative population of stem cells derived from mouse bone-marrow after transplantation. All the experiments conducted were approved by the Institutional ethical committee.

Disclosures: G. Minhas: None. **S. Prabhakar:** None. **A. Anand:** None.

Poster

250. **Ischemia: Animal Models**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 250.03/AA11

Topic: C.09. Ischemia

Support: SNUBH Research Fund 03-2011-006

Title: Functional connectivity between the cerebellum and the cerebral cortex related to higher brain function

Authors: *S. JUNG[1], Y. KIM[2], N.-J. PAIK[3];
[1]Rehabil. Med., [2]Nuclear Med., Seoul Natl. Univ. Boramae Med. Ctr., Seoul, Korea, Republic of; [3]Rehabil. Med., Seoul Natl. Univ. Bundang Hosp., Seongnam-Si, Gyeonggi-Do, Korea, Republic of

Abstract: Background
This study is to investigate whether there is functional connectivity between the cerebellum and the cerebral cortex and whether this connectivity can be used as a therapeutic application for cognitive improvement.
Methods
The intracerebellar Endothelin-1 (Et-1) injection was used to elicit focal cerebellar ischemia in male Sprague-Dawley rats. Et-1 was injected stereotaxically in the left posterior cerebellar hemisphere and in the left vermis. Repetitive transcranial magnetic stimulation (rTMS) was applied to the right cerebral cortex at post-ischemia day 7 over a 2-week period. Rats were randomly assigned to 20Hz frequency rTMS (a total of 1,600 impulses), 1Hz frequency rTMS (a total of 1,200 impulses), and sham rTMS group. Functional outcome regarding motor and cognitive function was measured before and after rTMS. Furthermore, protein expression was determined using Western blot. We performed [18F]FDG PET scans 1, 7, 14, 30 days after after the administration of Et-1.
Results
The intracerebellar Et-1 injection caused neurological deficit. Although there was no significant difference in the improvement of motor function among rTMS groups, both 1Hz and 20Hz rTMS group after left vermis infarction showed stastistically significant improvement in the attention and the memory. Phospho-pkc and mGluR1α decreased in the left cerebellar hemisphere of 1Hz and 20Hz rTMS group after the left cerebellar posterior hemisphere infarction. Synaptophysin increased in the right cerebral cortex of 20Hz rTMS group after the left cerebellar posterior hemisphere infarction. Phospho-pkc increased in the right cerebral cortex of 20Hz rTMS group after the left vermis infarction. pkc increased in the right cerebellar hemisphere of 20Hz rTMS group and decreased in the right cerebellar hemisphere of 1Hz rTMS group after the left vermis infarction. mGluR2 decreased in the right cerebral cortex of 1Hz and 20Hz rTMS group after the

left vermis infarction.

Conclusions

Therapeutic effects of rTMS applied to contralateral cerebral cortex after the vermis infarction suggests that there may be functional cerebro-cerebellar connectivity.

Disclosures: S. Jung: None. **Y. Kim:** None. **N. Paik:** None.

Poster

250. **Ischemia: Animal Models**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 250.04/AA12

Topic: C.09. Ischemia

Support: Bourse non-FRIA (ULg)

Title: Transient MCAO prevents habit formation in C57Bl/6J mice

Authors: J. LINDEN[1], A. FERRARA[3], M. BASTIN[3], *J.-C. L. PLUMIER[2];
[1]Psychologie: cognition et comportement, [2]Univ. De Liège, Liège, Belgium; [3]Univ. de Liège, Liège, Belgium

Abstract: Pathologies affecting the striatum (e.g., Parkinson's and Huntington's disease) can result in impaired habit learning abilities. Likewise, such impairments have also been observed after stroke affecting the middle cerebral artery territory (encompassing the striatum). However, habit learning has never been investigated in animal stroke models, for which it could be a reliable measure of cognitive deficits. We thus assessed the ability to learn a habitual sequence of lever-presses using operant conditioning in mice after MCAO, one of the most common stroke models.

C57Bl/6J mice underwent MCAO or sham surgery. Sensorimotor functioning was assessed using the vertical pole test, rotarod and amphetamine-induced rotation test. Habit learning was evaluated using the operant serial order learning (SOL) task: mice had to perform a series of two consecutive lever-presses (i.e., left then right) to obtain a food reward. Lesion extents were finally determined using anti-NeuN immunohistochemistry.

MCAO mice were significantly impaired in both the rotarod and vertical pole test, and displayed a significantly greater number of ipsilateral rotations after amphetamine administration. In the operant SOL task, MCAO committed more errors than sham; moreover, they did not show any significant increase in performance along the sessions. Histological analysis showed consistent striatal and cortical infarctions.

The lack of habit learning ability in MCAO mice is congruent with both the literature

investigating the effect of striatal lesion in animals and the symptomatology observed in human stroke patients. Habit learning could thus be regarded as a reliable measure of functional outcome after MCAO, in combination with test assessing sensory and motor aspects.

Disclosures: J. Linden: None. **J.L. Plumier:** None. **A. Ferrara:** None. **M. Bastin:** None.

Poster

250. **Ischemia: Animal Models**

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Program#/Poster#: 250.05/AA13

Topic: C.09. Ischemia

Support: NIH-NINDS NS-066001

NIH-NINDS NS-055832

1T32DC010775-01

Title: The spread of sensory-evoked cortical activity along long-range horizontal projections is critical to complete protection from ischemic stroke following permanent MCA occlusion

Authors: R. FROSTIG, *C. C. LAY, N. JACOBS, P. VU, M. DAVIS;
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Abstract: Using a rodent model of ischemic stroke (permanent middle cerebral artery occlusion; pMCAO), previous research has shown that tactile or auditory stimulation, delivered within 2 hours of ischemic onset can confer complete protection from impending stroke by re-directing blood flow through collateral vessels. How can sensory stimuli, such as whisker stimulation or white noise, protect such a large area of cortical MCA territory (including motor, somatosensory, auditory cortices)? Blood flow redirection is clearly necessary for protection, but it may not be sufficient. Prior research in our lab has demonstrated that sensory stimulation results in a large spread of evoked intracortical subthreshold activity supported by long-range intracortical horizontal projections directly connecting unimodal primary cortices, and we hypothesized that such spread within the ischemic cortex is fundamental to protection from stroke. Using functional imaging, neuronal recording, blood flow imaging, and histological analysis, we tested the role of neuronal activity, by blocking the spread of auditory-evoked cortical activity via surgical transection of the gray matter between primary auditory, and primary somatosensory cortices. Experimental animals (n=10) received protective auditory stimulation immediately following pMCAO and transection, while sham-pMCAO controls received auditory treatment

following transection and sham-occlusion of MCA (n=10). Within control animals, the transection with sham-pMCAO did not disrupt function, blood flow, or tissue health in either the auditory or somatosensory cortex. Experimental animals also maintained baseline or greater levels of cortical function (evoked and spontaneous neuronal activity) and did not sustain infarct within the auditory cortex. Beyond the transection location, however, experimental animals demonstrated highly reduced somatosensory function and cortical infarct (38.2±6.9mm3). Interestingly, blood flow in both cortices returned to baseline levels. Thus, protection occurs specifically within the spread of evoked cortical activity which is supported by an underlying system of long-range horizontal projections, and regions outside the bounds of this spread remain vulnerable to ischemic stroke damage. Therefore, while sensory-evoked blood flow redistribution is a necessary prerequisite, the spread of evoked activity is also critical for complete protection following pMCAO. If applicable to humans, a greater emphasis upon the role of cortical activity during ischemia may lead to innovative treatment strategies that help protect the human brain from stroke.

Disclosures: R. Frostig: None. **C.C. Lay:** None. **N. Jacobs:** None. **P. Vu:** None. **M. Davis:** None.

Poster

250. **Ischemia: Animal Models**

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Topic: C.09. Ischemia

Support: Fundação Araucária

CAPES

CNPq

State University of Maringá

Title: Time course of behavioral and hippocampal neurogenesis after transient global cerebral ischemia in swiss mice

Authors: *L. M. SOARES, A. P. SCHIAVON, H. MILANI, R. M. M. W. DE OLIVEIRA; Farmacologia e Terapêutica, Univ. Estadual De Maringá, Maringá, Brazil

Abstract: Introduction: Hippocampal neurogenesis has been demonstrated to occur in response to transient global cerebral ischemia (TGCI), as a compensatory mechanism. However, the role

of hippocampal neurogenesis induced by TGCI in ischemic mice is not clear. The objective of this study was to characterize the time course of behavioral and hippocampal neurogenesis in Swiss mice subjected to TGCI. **Methods**: The procedures were approved by the Ethical Committee on Animal Research (004/2011). Male Swiss mice (30-40g) were subjected to TCGI by the two-vessel occlusion method. Seven, 14 or 28 days after reperfusion the animals were submitted to the Morris water maze (MWM), for evaluation of spatial memory, or to the open field (OF) and elevated plus maze (EPM), to access general locomotor activity and anxiety levels, respectively. Hippocampal neurodegeneration and neurogenesis were evaluated using Fluoro-Jade C (FJC) histochemistry and doublecortin (DCX) immunohistochemistry, respectively. Data, expressed as mean ± SEM, were analyzed by two-way ANOVA or Kruskall Wallis ANOVA (KW), followed by post hoc Duncan's or Dunn´s tests. **Results**: Ischemic animals presented an increase in the latency to find the platform as compared to controls (KW = 12.9, P = 0.005), 7 days after reperfusion (ischemic 466.8±36.1, control 337.1±22.0). ANOVA showed that ischemic animals presented a significant decrease in the 'number of entries in the center' (K-W=9.6, P=0.02) and 'time spent in the center' in the OF (K-W=10.9, P=0.01) 28 days after reperfusion (ischemic 13.5±4.1, control 26.7±2.5). In the EPM, ischemic animals presented a significant decrease in the '% of open arm entries' ($F_{3,39}$=4.5, P=0.009) and in the '% of time in the open arms' ($F_{3,39}$=9.2, P=0.0001) 7 and 14 days after reperfusion (7 days=7.9±2.70; 14 days=7.4±1.97; control=20.2±1.81). An increase of FJ-C positive cells (KW =13.3, P = 0.04) was detected at 7 (100.0±24.3) and 14 (16.8±10.2) days after ischemia. A decrease in the number of DCX-positive neurons was detected at 14 (209.1±30.2) and 28 days (188.8±20.2) following TGCI ($F_{3,24}$=19.7, P<0.0001). **Conclusions:** TGCI induced memory impairments at 7 days after reperfusion. Anxiety-related behaviors were observed at 7 days and sustained up to 28 days after reperfusion. Hippocampal neurodegeneration was detected at 7 and 14 days after reperfusion and decreased hippocampal neurogenesis was observed at 14 and 28 days after TGCI. Knowledge of the time course of hippocampal neurogenesis following TGCI has important implication on the assessment of neuroprotective drugs within a period when the intervention could change the ongoing ischemic processes and functional outcome.

Disclosures: L.M. Soares: A. Employment/Salary (full or part-time):; Fundação Araucária, Capes, CNPq. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); State University of Maringá.. **A.P. Schiavon:** None. **H. Milani:** None. **R.M.M.W. de Oliveira:** None.

Poster

250. **Ischemia: Animal Models**

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Topic: C.09. Ischemia

Support: NIH Grant NS051644

Title: Amelioration of motor dysfunction in the rat spinal air embolism model by human spinal stem cell implantation

Authors: *O. KAKINOHANA[1], M. HRUSKA-PLOCHAN[1], S. MARSALA[1], K. JOHE[2], J. DUMPIT[1], A. MIYANOHARA[1], M. MARSALA[1];
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Abstract: Background: In our previous study, we have developed a rat spinal air embolism model which displays comparable neurological dysfunction and spinal pathology as seen in human patients with decompression
sickness. Using this animal model, we now explored the treatment effect of spinal grafting of human fetal spinal stem cells (hSSCs) (Neuralstem, Inc., MD) in rats with fully developed spinal air embolism-induced spinal injury. Methods: To induce spinal air embolism, male SD rats (n=18) were anesthetized with isoflurane and a 2F Fogarty catheter was placed into the descending aorta from the left femoral artery with the balloon tip of the catheter resting just above the truncus coeliacus. The PE-10 catheter was placed from the left carotid artery to the descending thoracic aorta to reach the Th7-8 level. After heparinization (200 IU), the balloon was inflated (50 µl) and air (200 µl/kg BW) was injected through the PE-10 catheter at a rate of 50 µl/min. After air injection, the balloon was deflated, catheters were removed and animals were allowed to recover. Two weeks after surgery, rats received spinal lumbar (L2-L6) injections (20 bilateral injections, 30,000 cells/µl/inj) of GFP-labeled human spinal stem cells (RSC566, Neuralstem) and survived for 3 months. All animals were continuously immunosuppressed with Tacrolimus (3 mg/kg/day). Recovery of neurological function was assessed using BBB score and Hoffman-reflex. Before sacrifice, descending motor tracts (corticospinal, rubrospinal) and primary afferents (L2-L6) were labeled with AAV9-syn-dsRED and cholera toxin B subunit, respectively. At 3 months, animals were perfused with 4% PFA. The presence of grafted human cells was validated by GFP+ fluorescence and combined with staining with human-specific (hNUMA, HO14, hNSE, hSYN) and non-specific (DCX, MAP2, Chat) antibodies and analyzed with confocal microscopy. Results: Animals receiving spinal grafts of hSSCs that showed grafted cell survival had progressive improvement in BBB score (6.8±0.6 ◊ 14.8±6.8), which was significantly higher than seen in media-injected animals or in animals with no graft survival (4.7±0.5 ◊ 5.5±1.0; $p<0.05$). No significant differences in H-reflex were seen. Grafted human stem cells showed extensive axo-dendritic sprouting and expression of neuronal (DCX, NSE, NeuN) and non-neuronal (GFAP, Olig2) markers. Terminals of descending motor tracts were found in the vicinity of grafted human neurons. Conclusion: These data indicate that spinal grafting of human spinal stem cells can represent an effective treatment modality to ameliorate neurological dysfunction associated with the spinal form of decompression sickness.

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Poster

250. **Ischemia: Animal Models**

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Topic: C.09. Ischemia

Title: SIRT1 plays a potent protective role against chronic cerebral hypoperfusion

Authors: Y. HATTORI[1,2], *M. IHARA[3], N. OISHI[4], Y. OKAMOTO[5], K. NAGATSUKA[6], R. TAKAHASHI[1], H. FUKUYAMA[4], M. KINOSHITA[7];
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Abstract: **Background—**SIRT1 is the mammalian homologue of yeast silent information regulator-2, a member of the sirtuin family of protein deacetylases which have gained increasing attention as mediators of lifespan extension in several model organisms. Previous reports have suggested a protective role of SIRT1 in neurodegenerative diseases and cardiac ischemic/reperfusion injury. We here examined whether SIRT1 is protective against chronic cerebral hypoperfusion in rodents.
Methods and Results—Mice overexpressing SIRT1 under the control of mouse prion promoter and their wild-type littermates were subjected to bilateral common carotid artery stenosis (BCAS) using external microcoils. Laser speckle flowmetry and ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) showed significant preservation of cerebral blood flow (CBF) and metabolism in the SIRT1-overexpressing mice compared to the wild-type littermates following BCAS. Prior administration of
eNOS-inhibitor, caveollin-1 scaffolding domain peptide, abolished the above CBF-preserving effect of SIRT1-overexpressing mice, suggesting that eNOS had a direct role in the CBF preservation by SIRT1. The level of reactive oxygen species labeled for 8-hydroxy-2'-deoxyguanosine in the cerebral vessels was dramatically reduced while cerebral expression of MnSOD was significantly upregulated in SIRT1-overexpressing mice at 2 hours after BCAS. As

a result, SIRT1-overexpressing mice at one month after BCAS showed significantly less impairement in the brain metabolism (as assessed with FDG-PET), the white matter integrity (Klüver-Barrera
staining), and the spatial working memory (8-arm radial maze test) compared to the wild-type littermates. Intraperitoneal administration of resveratrol (SIRT1 activator: 100mg/kg body weight x 7 days prior to BCAS) significantly preserved CBF after BCAS compared to the vehicle administration.
Conclusion—These results suggest that SIRT1 is a promising therapeutic strategy against cerebral hypoperfusion due to the antioxidative activity, the eNOS-associated vasodilation, and the resultant CBF preservation.

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Poster

250. **Ischemia: Animal Models**

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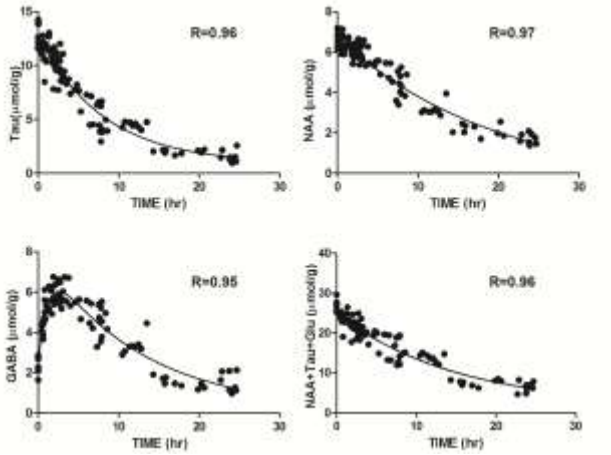
Title: Non-invasive biomarkers estimate the time of onset of cerebral ischemia

Authors: C. BERTHET[1], L. XIN[2], C. BENAKIS[1], *R. GRUETTER[2,3,4], L. HIRT[1,4], H. LEI[2,3]; [1]Dept. of Clin. Neurosciences, Ctr. Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [2]EPFL, Lausanne, Switzerland; [3]Univ. of Geneva, Geneva, Switzerland; [4]Univ. of Lausanne, Lausanne, Switzerland

Abstract: Thrombolysis, the only currently available treatment for ischemic stroke can be administered only in a narrow time window of 4.5 hours. A significant number of patients have an unknown time of onset as the stroke occurred during their sleep, which disqualifies them for thrombolysis. The objective of this study was to determine whether magnetic resonance spectra

of the ischemic striatum could provide useful information for the estimation of the onset time of cerebral ischemia.
We modeled ischemic stroke in male ICR CD1mice using a permanent middle cerebral artery filament occlusion model with laser Doppler control of the regional cerebral blood flow (<20% during ischemia). Mice were then measured by repeated magnetic resonance spectroscopy of the ipsilateral striatum in a 14.1T system. The acquired MR spectra were processed and quantified referencing to striatal water contents, as previously described for transient cerebral ischemia (Lei H et al. 2009, Berthet C et al. 2011) yielding metabolite concentrations.
We observed different spectral patterns after permanent ischemia than after transient ischemia, with an initial striking increase in gamma-aminobutyric acid (GABA) and no increase in glutamine. We observed a mono-exponential decline (coefficient of determination, R2>0.90) of e.g. taurine (Tau), N-acetylaspartate (NAA) and the sum of Tau, NAA and glutamate (Glu, as in figure). Using these characteristic reductions, in a set of blinded measurements we were able to estimate the time of onset of permanent ischemia with an accuracy of approximately +/- 30 min (SDs).
This is a novel approach, in mice, addressing the clinically highly relevant problem of determining the time of onset of ischemic stroke in stroke patients.



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Poster

250. **Ischemia: Animal Models**

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Topic: C.09. Ischemia

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Title: Chronic cerebral hypoperfusion results in distinct behavioral and pathological outcomes in normotensive and hypertensive rats

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Abstract: Vascular dementia (VaD) is the second most common form of dementia. VaD is often caused by chronic subcortical ischemia accompanied by white matter stroke where ischemic demyelination and axonal damages are observed. A lack of adequate animal model for VaD prevents systematic studies into pathomechanism of chronic subcortical ischemia and resultant white matter injury. Chronic cerebral hypoperfusion by steno-occlusion of one or both carotid arteries has been frequently employed in rodents to induce white matter stroke. However, reported pathological findings varied and the extent of white matter injury has been often modest at best. This study was undertaken to test a hypothesis that chronic cerebral hypoperfusion to rats with underlying hypertension may result in more pronounced white matter injury. We compared neurobehavioral and neuropathological features between normotensive (NR) and spontaneously hypertensive rats (SHR) in Wistar background after chronic cerebral hypoperfusion induced by bilateral common carotid artery occlusion (BCCAO). There was no difference in neurobehavioral measures between NR and SHR before BCCAO. SHR exhibited obvious spatial memory deficit after BCCAO, while spatial memory was intact in NR. While performance in delayed alternation task was worsened to a similar extent after BCCAO in both groups,,only SHR showed dimished ability in novel object recognition test after BCCAO but not NR. Neuropathological evaluation in white matter and hippocampus showed increased blood-brain barrier disruption in white matter and increased inflammation in hippocampus in SHR than WR. Differences in oligodendrocytes damage and myelination are being examined. The results of our study will provide important information on whether rodents with underlying hypertension would be more susceptible to chronic cerebral ischemia in developing white matter ischemic damages as is often observed in human patients.

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Poster

250. **Ischemia: Animal Models**

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Topic: C.09. Ischemia

Support: NIH grant NS073779

Title: Post-ischemic mitochondrial dysfunction in brains of diabetic rats exposed to recurrent hypoglycemia

Authors: P. FUCHS, F. DIAZ, *K. DAVE;
Neurol., Univ. Miami Sch. Med., MIAMI, FL

Abstract: Stroke and heart disease are the most serious complications of diabetes. The major side effect of intensive therapy to control blood glucose levels in both type 1 and type 2 diabetics is recurrent hypoglycemic (RH) episodes. Prior exposure to RH exacerbates cerebral ischemic damage in insulin-treated streptozotocin-diabetic rats. The present study investigates the nature of post-ischemic mitochondrial dysfunction in RH-exposed diabetic rats, as mitochondrial dysfunction plays an important role in cerebral ischemic damage. Streptozotocin-induced diabetic rats were used as an animal model. Four experimental groups were examined: 1) naïve (non-diabetics), 2) insulin-treated diabetics (ITD), 3) ITD + RH (diabetics on insulin therapy experiencing RH), and 4) ITD + RH + Glucose (control for additional insulin injected to induce hypoglycemia). RH was induced once a day for five days. Global cerebral ischemia (8 min, 2VO + hypotension) was induced the day after the last hypoglycemia treatment. Hippocampal mitochondrial function was assessed overnight after induction of global cerebral ischemia. Earlier, presented in abstract forms, we observed that the rate of oxygen consumption in the presence of pyruvate and malate was lower in the ITD + RH group compared to naïve (25%, $P<0.05$), ITD (46%, $P<0.001$), and ITD + RH + Glucose (33%, $P<0.001$) groups. Complex I activity was lower in the ITD+RH group compared to naïve (36%, $P<0.001$), ITD (38%, $P<0.001$), and ITD + RH + Glucose (33%, $P<0.01$) groups. The decrease in Complex I activity could be due to a decrease in the steady-state levels of the fully assembled complex, deficits in the assembly or stability of the complex, or posttranslational modifications such as phosphorylation. Currently, we are investigating the molecular mechanisms responsible for the decrease in Complex I activity using blue native gel electrophoresis and western blotting to assess the assembly status of this respiratory complex. Our results suggest that enhanced post-ischemic mitochondrial dysfunction at the level of Complex I may be responsible for exacerbated cerebral ischemic damage in in RH-exposed diabetic rats. Understanding the mechanism by which RH exposure increases cerebral ischemic damage may help lower the severity of ischemic damage in diabetic patients.

Disclosures: P. Fuchs: None. **F. Diaz:** None. **K. Dave:** None.

Poster

250. **Ischemia: Animal Models**

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Program#/Poster#: 250.12/BB2

Topic: C.09. Ischemia

Title: Increased hemorrhage and mortality rate after transient focal ischemia in a genetic mouse model of type 1 diabetes

Authors: *A. K. LAI, A. C. LO;
Ophthalmology, The Univ. of Hong Kong, Hong Kong SAR, China

Abstract: PURPOSE: Epidemiological studies showed that type 1 diabetic patients have a higher risk of cerebrovascular mortality from stroke. Amongst those who survived after stroke, the median survival is only half as compared with those in the general population. It has been suggested that type 1 diabetes is a risk factor for stroke; however, the underlying mechanisms are still not clear. In the current study, we aim to elucidate the potential mechanisms contributing to the exacerbation.
METHOD: $Ins2^{Akita/+}$ mice, a type 1 diabetic murine model, and their wildtype ($Ins2^{+/+}$) littermates at 12 weeks of age were challenged with experimental stroke induced by middle cerebral artery occlusion (MCAO) (2h ischemia and 22h reperfusion). Mortality rate was calculated at 1, 2, 4, and 22h of reperfusion and neurological deficits were accessed by the end of the 22h-reperfusion. Brain slices were prepared and stained with 2, 3, 5-triphenyltetrazolium chloride for estimating the infarct area, infarct volume, hemispheric swelling, and hemorrhagic area. Immunohistochemical experiments using antibodies against immunoglobulin G, aquaporin 4 and occludin were performed on brain sections to compare blood vessel integrity.
RESULTS: A higher mortality rate and a shorter survival period were observed in $Ins2^{Akita/+}$ mice after the MCAO challenge when compared with $Ins2^{+/+}$ mice. In the survived $Ins2^{Akita/+}$ mice, hemorrhage was observed in almost every individual (90%). The hemorrhagic areas were remarkably increased in $Ins2^{Akita/+}$ mice, with the majority localizing in the infarct core while a small proportion residing in the penumbra. However, no significant difference was found in neurological deficits, infarct area, infarct volume or hemispheric swelling when compared with $Ins2^{+/+}$ mice. Immunohistochemistry results showed similar staining pattern and intensity of proteins associated with blood vessel integrity, including immunoglobulin G, aquaporin 4 and occludin, in both $Ins2^{Akita/+}$ and $Ins2^{+/+}$ mice.
CONCLUSION: We showed that induction of MCAO in $Ins2^{Akita/+}$ mice could mimic the clinical observations of high mortality and shortened survival in type 1 diabetic patients upon stroke. The

presence of hemorrhage, which was associated with blood vessel leakage and extravasation, may be a potential cause of the exacerbation in the injured brains. The similarities in infarct size, hemispheric swelling as well as blood vessel integrity may possibly be due to a selection of surviving animals that were relatively more resistant to ischemic insult.

Disclosures: A.K. Lai: None. **A.C. Lo:** None.

Poster

250. **Ischemia: Animal Models**

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Topic: C.09. Ischemia

Support: Acorda Therapeutics, Inc.

Title: Measures of long-term neurological recovery differentially affected by stroke severity in an intraluminal filament MCAO model in adult rats

Authors: *C. MCEWEN, S. J. E. WONG-GOODRICH, Z. HUANG, E. L. TROY, A. GANGULY, R. W. COLBURN, A. O. CAGGIANO, T. J. PARRY;
Res. and Develop., Acorda Therapeut., Ardsley, NY

Abstract: Ischemic stroke induced by occlusion of cerebral vasculature in humans leads to long-term disability and mortality. Depending on the neuroanatomical location of the stroke, many individuals experience devastating loss of motor function, sensory function, aphasia, visual disorders, and/or memory loss. Middle cerebral artery occlusion (MCAO) in rats is the most commonly used method to study and assess potential therapies for cerebral ischemic stroke. Although some reports have described chronic neurological deficits following intraluminal filament MCAO in rats with modest infarct size (Lindner et al., 2003), the goal of this study was to evaluate long-term neurobehavioral recovery at higher temporal resolution using a filament modified to produce a greater degree of infarct severity. Baseline assessment of neurological function was conducted on adult male Wistar rats, using the forelimb/hindlimb placing, body swing, ledged beam, and cylinder tests. Rats were then subjected to MCAO for 1 or 2 h via the intraluminal filament method. Sham animals were also included. To assess functional recovery, all rats were retested on 1, 3, and 7 days and then weekly following sham or MCAO surgery. At 9 weeks post-surgery, rats were then perfused and brains harvested for histological analyses. In a subset of animals, infarct size was also examined as a function of occlusion time. Coronal brain sections were collected throughout the extent of the brain, stained with hematoxylin and eosin, and percent infarct size was recorded for each rat at each section interval. Analyses of behavioral

scores on each test revealed significantly different recovery profiles between 1 h and 2 h MCAO groups. Notably, 2 h MCAO rats exhibited a more protracted recovery in forelimb placing, body swing, and cylinder tests through 9 weeks post-injury. Through 14 days post-MCAO, the rate of recovery of forelimb placing for 2 h MCAO rats was significantly slower than 1 h MCAO rats (slopes = 0.26 ± 0.06 and 0.62 ± 0.89 for 2 h and 1 h, respectively). In contrast, both groups had similar recovery profiles on the hindlimb placing and ledged beam tests. Analysis of infarction (% of contralateral hemisphere) revealed that rats subjected to 2 h MCAO had almost twofold larger infarct areas (41.80 ± 4.58%) compared to 1 h MCAO rats (22.1% ± 3.81, $p < 0.05$). Analysis of specific neuroanatomical correlates differentially affected by stroke severity as well as immunolabeling of neuroplasticity markers is ongoing.

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Poster

250. **Ischemia: Animal Models**

Location: Halls B-H

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Program#/Poster#: 250.14/BB4

Topic: C.09. Ischemia

Title: Introduction of an alternative focal cerebral ischemia model in adult zebrafish by photothrombosis

Authors: *X. YU[1], Y. LI[2];
[1]Biol. Sci., [2]Biomed. Sci., Ohio Univ., Athens, OH

Abstract: As the more commonly used models (suture model and distal middle cerebral artery occlusion) for focal cerebral ischemia are often technically challenging, using simpler models to induce a predictable focal ischemic lesion have been developed as an alternative. One of these methods consists of the photothrombosis, which is induced by the illumination of the brain after the systemic delivery of a photosensitive dye, Rose Bengal. Rose Bengal releases singlet oxygen, which breaks the endothelial cells of the blood vessel under light exposure, and triggers the coagulation pathway in the location of the irradiated tissue. In the past decades, the zebrafish (Danio rerio) is widely used to model human diseases, including neurological disorders and in stress research. In the present study, we used the adult zebrafish as a model of focal cerebral ischemia. Zebrafish with the body weight ranging from 0.3 g to 0.5 g were used in the experiment. After anesthesia, Rose Bengal was systemically delivered to zebrafish by intraperitoneal injection according to the body weight (50 μg/g, 100 μg/g and 200 μg/g). The zebrafish was then placed into a self-designed chamber in the upright posture with continuous water and O2 perfusion. Cold light probe was placed right above the optic tectum region of the zebrafish brain. The zebrafish was covered with only the optic tectum exposed for different periods of time (5 minutes, 10 minutes, 20 minutes and 30 minutes). Rose Bengal treated zebrafish without light exposure behaved normally. However, the zebrafish after light exposure exhibited abnormal swimming patterns or movement behaviors. The recovery and behavioral changes after photothrombosis were recorded daily for 3 days. The mortality rate of each group was also measured. The results showed the photothrombosis effect is dose-dependent with Rose Bengal and light exposure. The optimal dose of Rose Bengal (100 μg/g) and light exposure (intensity of 800 μW/cm2 for 30 minutes) yielded significant brain damage. After treatment, 41% of the treated zebrafish exhibited abnormal swimming patterns such as circling and rotating swim, which were related to the damage of the optic tectum. Among those abnormal swimming zebrafish, 78% of them died subsequently. In addition, 27% of the zebrafish died after treatment without showing circling or rotating swim pattern. Quantified TTC (2,3,5-triphenyltetrazolium chloride) staining showed the significant damage of the optic tectum on photothrombotic treated zebrafish. Data suggest that Rose Bengal, with light exposure induces reproducible photothrombotic brain damage on zebrafish, and that zebrafish can be used as a model of focal cerebral ischemia.

Disclosures: X. Yu: None. **Y. Li:** None.

Poster

250. **Ischemia: Animal Models**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 250.15/BB5

Topic: C.09. Ischemia

Support: NIH/SCTR UL1 RR029882

NIH Grant C06 RR015455

Title: Diffusional kurtosis imaging (DKI): A potential novel MRI biomarker of acute stroke and rehabilitation-induced neural plasticity

Authors: *R. WEBER[1], E. S. HUI[2,3], J. H. JENSEN[2,3], X. NIE[2,3], M. F. FALANGOLA[1,2,3], J. A. HELPERN[1,2,3], D. L. ADKINS[1];
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Abstract: Currently, there are only limited prognostic tools or biomarkers to determine who will recover motor functions and who will respond best to rehabilitation treatments following stroke. Magnetic Resonance Imaging (MRI) does reveal some common characteristics in brain activation patterns that indicate level of motor recovery following stroke and has been shown, to a limited degree, to reveal training-induced changes in cortical thickness. Advanced diffusional MRI (dMRI) techniques, such as diffusional kurtosis imaging (DKI), are highly sensitive to microstructural changes in brain and provide unique information about white matter connectivity and integrity and thus may be of great prognostic value However, the application of these techniques to stroke has only recently been investigated and a clearer understanding of the relationships between novel dMRI metrics, tissue biophysics and morphology, and behavioral outcomes following acute stroke and post-injury rehabilitative training is needed. In this study, we investigated the relationship between DKI metrics and peri-lesional alterations in glia, neurons and axons; we related these findings to stroke- and rehabilitation-induced sensory and motor recovery. Following unilateral endothelin-1 ischemic lesion of the sensorimotor cortex, adult Long-Evans male rats (3 - 4 mo) underwent sensitive behavioral assessments and were imaged pre-injury and at 2, 24, or 72 h post-surgery, using a 7T Bruker Biospec MRI scanner. Diffusion-weighted images were acquired with 3 b-values (650, 1300, 2000 s/mm^2) along 30 directions using TR/TE=4750/32.5ms, matrix=128x128, resolution=0.23x0.23x1mm^3, and NEX=2. Diffusion and kurtosis tensors were calculated with Diffusional Kurtosis Estimator (DKE) and Cerebral Microenviroment Modeling (CMM) parameters, a new analysis technique,

were computed using in house C and MATLAB programs. Multi-slice regions-of-interest (ROIs) were manually drawn in the infarct (ipsilateral) and contralateral hemisphere. The mean diffusivity (MD), mean kurtosis (MK), and fractional anisotropy (FA) revealed longitudinal changes following stroke. Consistent with a previous study, MK of the infarcted tissue remains higher than normal during the first 72hr post-ischemia, although MD pseudonormalized. It is possible that the sustained increase in MK is due to axon beading, reactive gliosis or cellular oedema. Ongoing analysis will provide further insight into the pathophysiological events underlying the change in DKI metrics during acute and early subacute phases. These data will be further related to chronic alterations induced by motor rehabilitative training.

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Poster

250. **Ischemia: Animal Models**

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Topic: C.09. Ischemia

Support: NSERC grant

CIHR grant

Title: Visualizing ganglioside expression using MALDI imaging mass spectrometry in a rat model of beta amyloid toxicity and stroke

Authors: *S. CAUGHLIN, J. D. HEPBURN, D. H. PARK, D. F. CECHETTO, S. N. WHITEHEAD;
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Abstract: Stroke and Alzheimer's disease (AD) are often co-morbid conditions that show synergistic damaging effects within the brain. Previous experimental work has demonstrated that in the presence of beta amyloid (Aβ) stroke infarct size grows over time, however clear mechanisms mediating this observation are unknown. Gangliosides, a member of the glycosphingolipid family which are enriched in the central nervous system, may play a role in mediating this synergism as their expression is altered in both AD and stroke. Gangliosides have a heterogeneous expression profile within the brain. Complex gangliosides that contain longer oligosaccharide chains and more sialic acid residues such as GD1 and GM1 can be degraded to smaller, simpler ganglioside species such as GM2 and GM3. The degradation of complex

gangliosides in AD and stroke may lead to neurodegeneration. Here we examine the expression of various ganglioside species in the striatum of rats using Matrix-Assisted Laser Desorption/Ionization (MALDI) Imaging Mass Spectrometry (IMS). MALDI IMS enables the visualization of molecule distribution within intact tissue sections and also produces corresponding mass spectra. Each species of ganglioside can distinguished based on their mass-to-charge ratio and can be imaged simultaneously in a single scan. 3 month old male Wistar rats were either given a unilateral striatal injection of endothelin-1 (stroke condition), an endothelin-1 injection and bilateral intracerebroventricular (ICV) injections of Aβ (25-35) (combination Aβ and stroke condition), injection with endothelin-1 and the non-toxic reverse Aβ fragment (35-25), or underwent sham surgery. Ganglioside expression was examined via MALDI IMS at both 3 and 21 days after treatment. With or without Aβ, GM1 was decreased within the striatum at 3 and 21 days following stroke compared to control. Conversely, with or without Aβ, GM2 and GM3 expression were increased within the striatum 3 days post stroke. Interestingly, by 21 days following stroke, only GM3 remained elevated within the striatum of combined Aβ/stroke rats and not stroke alone rats. Overall, increases in simple ganglioside species were observed immediately after stroke indicating that these potentially toxic gangliosides play an important role in stroke pathology. Over time, the accumulation of GM3 in the periphery of the stroke region observed only in the Aβ/stroke condition may be indicative of a mechanism of pathological synergism between AD and stroke.

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Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: C.09. Ischemia

Support: KAKENHI 24390016

KAKENHI 25460098

Title: TRPM2-mediated NO production in microglia/macrophage contribute to the progression of cerebral ischemic injury in mice

Authors: S. SAKIMOTO, H. SHIRAKAWA, K. ASAKURA, M. MUNAKATA, T. NAKAGAWA, *S. KANEKO;
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Abstract: Ischemic stroke is the third leading cause of death worldwide. Emerging evidence indicates that the activation of inflammatory cells in ischemic brain tissue can extend the brain infarction, but the activation mechanisms of the inflammatory cells remain to be elucidated. Transient Receptor Potential melastatin 2 (TRPM2), a Ca2+ permeable nonselective cation channel, is functionally expressed in the brain and immune cells, implying that TRPM2 could be involved in neuroinflammation during cerebral ischemia. Because of a lack of selective pharmacological blockers, the pathophysiological role of TRPM2 in ischemic brain damage has not been well-defined. In this study, we investigated the involvement of TRPM2 in transient focal cerebral ischemia using wildtype (WT) and TRPM2-knockout (KO) mice. Cerebral infarction and neurological deficits in WT mice were gradually aggravated after reperfusion whereas those in KO mice were not. Immunohistochemical analysis revealed that the migration of microglia/ macrophage in ischemic boundary zone was reduced in KO mice. In addition, the minocycline, an inhibitor of microglia/ macrophage activation, treatment prevented ischemic neuronal injuries in WT mice, but not KO mice. To explore the underlying mechanisms in TRPM2-KO mice. We generated bone marrow chimeric mice by transplanting bone marrow from GFP-wild-type or GFP-TRPM2-KO mice into irradiated recipients of both genotypes. Analysis of chimeric mice revealed that both central and peripheral deficiency of TRPM2 improved neurological deficits, suggesting that both microglia and macrophages play important roles in cerebral ischemia. Next, we focused on inducible nitric oxide synthase (iNOS) and found that administration of a selective iNOS inhibitor 1400W prevented ischemic neuronal injuries in WT mice, but not TRPM2-KO mice. Moreover, in vitro experiments demonstrated that the production of nitric oxide induced by toll-like receptor agonist, lipopolysaccharide and lipoteichoic acid, was markedly reduced in microglia as well as macrophages derived from TRPM2-KO mice. These results indicate that TRPM2 could mediate the cerebral ischemic injury through NO release from both microglia and macrophage.

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Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

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VA Merit

VA CDA

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Uehara Foundation

Title: Microglial TREM2 promotes neuronal phagocytosis after oxygen-glucose deprivation

Authors: R. KACIMI[1], T. M. KAUPPINEN[1,3], C. CALOSING[1], Z. ZHENG[1], C. L. HSIEH[2], M. NAKAMURA[2], *M. A. YENARI[4];
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Abstract: Triggering receptor expressed by myeloid cells-2 (TREM2) is a surface receptor present on microglia and macrophages. It was first described as a receptor of the innate immune system which bound pathogens and led to their phagocytosis. However, TREM2 was also found to bind an as yet undefined receptor in brain cells, particularly injured neurons. TREM2 deficiency leads to dementia. Thus, TREM2 may be an important molecule in the clearance of injured cells paving the way towards recovery and repair. We tested the hypothesis that TREM2 facilitates post-ischemic phagocytosis of ischemic brain cells, and limits the extent of injury caused by excessive inflammation. Using in vitro models of ischemia-like injury, we explored the potential contribution of TREM2 in neuron death and phagocytosis, and attempted to identify its ligand. We first studied Neuro2a cell cultures in combination with BV2 microglial. We confirmed the presence of TREM2 expression in microglial cells. When fluorescently labeled Neuro2a cells were co-cultured with BV2 cells and exposed to oxygen glucose deprivation (OGD), BV2 cells were found to robustly phagocytose Neuro2a cells because they took up the dye used to label neurons. When TREM2 was silenced using siRNA, neuronal phagocytosis was decreased. Microglial treatment with IL-4 induced a M2 phenotype as determined by arginase 1 induction, but had little effect on TREM2 expression. LPS exposure decreased TREM2 expression, whereas PMA, by inducing a phagocytic phenotype, had a modest effect. Since HSP60 has previously been described as a ligand of TREM2, we applied recombinant HSP60 to BV2 cells, but failed to observe transformation to a phagocytic phenotype. To determine potential TREM2 ligands in our models, we used a fusion protein consisting of TREM2 linked to a human Fc receptor and through a modified ChIP assay, found that it bound DNA. Since nucleic acids may be released from ischemic cells, we then determined that supernatants from OGD exposed Neuro2a cells led to TREM2 signaling using a reporter cell line. Finally, we studied TREM2 in primary cell cultures consisting of microglia co-cultured with neuron-astrocyte cultures exposed to OGD. Ablation of microglial TREM2 expression reduced microglial transformation into amoeboid morphology and their tendency to cluster around neurons. TREM2 ablation in microglia did not affect overall acute neuron cell death, but neurons appeared more damaged when TREM2 was deficient, and there was accumulation of cell debris. These results

indicate that TREM2 plays a role in the phagocytosis of ischemic brain cells, and suggests that TREM2 signaling may occur through the binding of extracellular nucleic acids.

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Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

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Topic: C.09. Ischemia

Support: Junta de Castilla y León Ref. LE184A12-2

Title: Age differences in the inflammatory response at the vascular level induced by global cerebral ischemia in rat

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Abstract: Assays of global cerebral ischaemia by bilateral clamping of the carotid arteries for 15 minutes were carried out in 3- and 18-month-old male Sprague Dawley rats. Blood pressure was maintained in the range of 40-50 mm Hg during the clamping. After 48 hours of reperfusion (48I/R), brains were quickly removed and transferred to a stainless steel rat brain matrix to obtain one parasagital section (3 mm) of each hemisphere (1.0 to 4.0 mm from the medial line) at 4ºC. In each section, CA3 and CA1 hippocampal areas, as well as the cerebral cortex just dorsal to the hippocampus, were dissected, frozen and stored at -80ºC until used. Protein and RNA were isolated and used for antibody assays and real time PCR quantification. Main adhesion molecules related with the inflammatory response, I-CAM1, V-CAM1, P-selectin and E-selectin were studied. Both cerebral cortex and hippocampus presented similar response in the transcripts of all the molecules studied. I-CAM1 transcripts increased in both young and old injured animals with respect to those of the sham operated animals. In contrast, V-CAM1 transcripts increased in young injured animals but decreased in old injured animals. E-selectin transcripts increased in the old injured animals and decreased in young animals, however E-selectin protein presented different behavior between cerebral cortex and hippocampus in both young and old injured

animals. We only found significant increases in P selectin levels between young injured and young sham animals.
Our results show that 48I/R induces significant age-dependent changes in the mRNA levels of these molecules. The response observed supports the existence of age-dependent differences in the mechanisms of rolling and adhesion induced by 48I/R, and correlates with differences in the apoptosis at this time.

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Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

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Topic: C.09. Ischemia

Support: NIH PO1 AG022550

NIH PO1 AG027956

Title: T helper cell subsets and regulatory T cells in long-term survived experimental stroke mice

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Physiolgoy & Pharmacol., West Virginia University, Ctr. For Basic and Translational Stroke Res., Morgantown, WV

Abstract: Ischemic stroke is a devastating Central Nervous System (CNS) condition marked by ischemic brain cell death and breakdown of Blood Brain Barrier (BBB). BBB dysfunction promotes leakage of brain signals to the periphery, which initiates the activation of the peripheral immune system. During the acute stroke phase, the activation- and stroke damage-induced immune cell apoptosis could compromise the immune system resulting in the immunosuppression, systemic infection and septicemia. The immune system has to experience a long-term to recovery following acute stroke. However, it is unknown when and how the immune system is revived normal. We employed the murine experimental stroke model, middle cerebral artery occlusion (MCAO) in the combination with immunological technology to determine the periphery immune system repair following stroke. The T helper cell (Th) subsets response reflects the function of the immune system and we here analyzed Th1, Th2, and Th17

cytokine level in the peripheral blood and spleen T cells of mice 3-month after MCAO. The MCAO mice express lower Th1 cytokine IFN-γ (5.683 ± 0.9243 vs. 8.450 ± 0.5169, n=6, P=0.03) and Th2 cytokine IL-10 (0.35 ± 0.05 vs. 1.133 ± 0.236, n=6, P=0.01) in the blood compared to control mice. However, in the spleen, both IFN-γ and IL-10 have recovered to normal. Interestingly, IL-17 secreting T cells (marker of Th17) were lower in the spleen of MCAO mice but regained relative normal in the blood. Foxp3+ regulatory T cells remained unchanged in both blood and spleen of long-term survived MCAO mice compared to age-matched control mice. These novel observations suggest that the recovery of the immune system after stroke requires an extended period of time. These findings provide evidence supporting the need for recovery therapy of the immune system after stroke.

Disclosures: H. Hu: A. Employment/Salary (full or part-time):; Center for Basic and Translational Stroke Research, Department of Physiology & Pharmacology, Robert C. Byrd Health Sciences Center, West Virginia University. **X. Ren:** None. **J.W. Simpkins:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

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Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-0002316)

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Title: Restoration by hesperetin of tryptophan metabolic enzyme systems protects against ischemic brain injury in mice

Authors: *W. LEE, S. KIM, S. KIM, Y. FANG, S. BAE, C. KIM;
Dept of Pharmacol, and MRCITR, Pusan Natl. Univ. Sch. of Med., Yangsan, Gyeongsangnam-Do, Korea, Republic of

Abstract:
The tryptophan metabolic enzyme systems of indoleamine 2,3-dioxygenase (IDO) and tryptophanyl-tRNA synthetase (TrpRS), which are involved in L-tryptophan catabolism and its use in protein synthesis, respectively, are associated with the neuroinflammation and immune response to acute cerebral ischemia. Hesperetin, a citrus flavanone, is known to help prevent tissue damage from oxidative stress in the brain. In this study we investigated the mechanisms

involved in cerebroprotective action of heseperetin in connection with the tryptophan metabolic enzyme systems. Male C57BL/6 mice were anesthetized and subjected to photothrombotic cortical ischemia. Hesperetin was administered i.p. 1 h after ischemic insult. Posttreatment with hesperetin significantly reduced the infarct size including infarct area and volume. Ischemic insult markedly altered the tryptophan metabolic enzyme systems, i.e. an increase in the expression of IDO, CD11b, and CD11c, and a simultaneous decrease in that of TrpRS. Hesperetin significantly decreased the expressions of IDO, CD11b, CD11c, p-JAK2 and p-STAT1 via increasing the expression of TrpRS. Hesperetin significantly restored the tryptophan metabolic enzyme system, inhibiting JAK/STAT signaling activation. These results suggest that the cerebroprotective effects of hesperetin might be associated with the increase in the TrpRS expression as well as the suppression of the IDO expression.

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Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

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Topic: C.09. Ischemia

Support: NS 40516

Title: Triggering receptor expressed on myeloid cells 2 (trem2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke

Authors: *M. KAWABORI[1], Z. ZHENG[1], C. CALOSING, Ph.D.[1], C. HSIEH[2], M. NAKAMURA[2], M. YENARI[1];
[1]Neurol., [2]Med., VA Med. Center/UCSF, San Francisco, CA

Abstract: Clearing cellular debris after brain injury represents an important mechanism to re-attain tissue homeostasis and promote functional recovery. Triggering receptor expressed by myeloid cells-2 (TREM2) is involved in the innate immune system, and carries out surveillance functions by binding and phagocytosing pathogens. TREM2 is expressed on macrophages and microglia, and promotes the phagocytosis of apoptotic brain cells. TREM2 also has anti-inflammatory properties and may promote tissue repair without the damaging effects of inflammation. Deficiency of functional TREM2 leads to accelerated dementia, and mutations in the TREM2 gene have been linked to Alzheimer's. Here we explore the significance of TREM2

in a stroke model. TREM2-knockout (KO) and wildtype (WT) mice were subjected to permanent distal middle cerebral artery occlusion ischemic model to elucidate the precise mechanisms of the TREM2 in the ischemic stroke model. While phagocytosis and infarcted brain tissue resorption was observed in wild-type mice by 28d, no phagocytosis and little infarcted brain resorption was seen in TREM2 KO ($p < 0.01$). TREM2 KO mice also had worsened neurological recovery ($p < 0.05$), and decreased viable brain tissue in the ipsilateral hemisphere. Numbers of activated microglia and macrophages in KO mice was decreased compared to WT. These findings establish the relevance of TREM2 in phagocytosis of the infarcted brain and emphasize the importance of phagocytosis in neurological outcome following experimental stroke.

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Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

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Program#/Poster#: 251.07/CC1

Topic: C.09. Ischemia

Title: Postischemic exercise increases cell survival and modulates differently astrocyte and microglia functions in hippocampal dentate gyrus following global cerebral ischemia in Wistar rats

Authors: *G. A. LOVATEL, K. BERTOLDI, V. R. ELSNER, F. V. PIAZZA, C. BASSO, F. D. MOYSÉS, P. V. WORM, C. A. NETTO, S. MARCUZZO, I. R. SIQUEIRA;
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Abstract: The effect of exercise on glial cells activation and cell survival after global cerebral ischemia yet remains poorly understood. Here, we investigated the effect of both pre and postischemic treadmill exercise protocols (20 min/day during 2 weeks) on glial cells immunostaining and survival of BrdU+ cells in the hippocampus of Wistar rats submitted to global ischemia. It was observed an increase of area occupied by astrocytes in both dentate gyrus and stratum radiatum induced by global ischemia. Moreover, a synergistic effect between ischemia and exercise on area occupied by astrocytes was showed. Postischemic exercise partially reversed the ischemia-induced increase on area occupied by microglia. It was showed an increase of the survival of BrdU-positive cells in the in dentate gyrus induced by cerebral ischemia and the postischemic exercise potentiated this phenomenon. In conclusion,

postischemic exercise increases cell survival and modulates differently astrocyte and microglia immunostaining in hippocampal dentate gyrus following global cerebral ischemia in Wistar rats.

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Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

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Title: Interleukin-33 induces a peripheral Th2-type shift that is protective in a mouse model of cerebral stroke

Authors: *P. K. KORHONEN, K. M. KANNINEN, S. LEHTONEN, S. WOJCIECHOWSKI, S. LEMARCHANT, I. KIDIN, E. POLLARI, E. SAVCHENKO, J. KOISTINAHO, T. MALM; A.I. Virtanen Inst. For Mol. Sci., Kuopio, Finland

Abstract: Cerebral stroke is one of the leading causes of death and disability. Ischemia induces a massive Th1-shifted inflammatory response both in the brain and the periphery, contributing to the outcome of stroke. Th1-type responses are usually neurotoxic due to increased pro-inflammatory cytokines whereas Th2-type responses and anti-inflammatory cytokines, including interleukin-4 (IL-4), are generally beneficial. Interleukin-33 (IL-33) is a recently discovered cytokine that acts as a transcription factor, a secreted traditional cytokine and danger signal. IL-33 induces cells of the immune system to produce anti-inflammatory cytokines including IL-4, IL-5, IL-10 and IL-13, and guides undifferentiated T cells to become Th2-type cells.
The aim of this study was to test whether peripherally administered IL-33 could induce a shift in the inflammatory response towards the Th2 direction and whether this response would be protective in a mouse model of ischemic stroke. IL-33 was injected intraperitoneally to young Balb/c male mice either one week prior to permanent middle cerebral artery occlusion (pMCAo) or immediately after the injury. One group of mice also received an anti-IL-4 antibody. Neuronal

damage was imaged by magnetic resonance imaging (MRI) and the mice were sacrificed either 1 or 3 days post ischemia. Tissues and plasma were analyzed by immunohistochemistry, flow cytometry and RT-PCR.
Peripheral IL-33 treatment significantly reduced the lesion size both 1 and 3 days after ischemia regardless of whether the treatment was started prior to or after induction of the injury. The treated mice exhibited significantly higher IL-4 expression in plasma and spleen, and administration of the anti-IL-4 antibody partly prevented the IL-33 mediated protection. IL-33 treatment also reduced astrocytic activation in the peri-ischemic area of the brain.
Our results show that peripheral modulation of immune response by IL-33 to the beneficial Th2-type direction is protective against ischemic insult and may represent a novel therapy for cerebral stroke.

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Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

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Topic: C.09. Ischemia

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Canada Foundation for Innovation

Title: Increased ephrin expression by neutrophil and microglia in a rat model of small-vessel stroke

Authors: *F. S. CAYABYAB[1], K. GOWRIBAI[2], W. WALZ[2];
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Abstract: In a modified pial vessel disruption (PVD) protocol used as a rat model of small-vessel stroke, increased expression of matrix metalloproteinases-2 and -9 (MMPs) contributes to the formation of lacuna-like cavity. Minocycline injections prevented the cavitation by inhibiting major expressions of MMPs from microglia/macrophages. However, we also observed MMPs in cells that did not label with microglial markers. Here we further characterized the cellular sources of MMPs that could regulate cavity formation. Inside the PVD-induced lesion sites, we found MMP-expressing cells with multi-lobulated nuclei that did not label with the microglia

markers ED1or OX-42. We hypothesized that these are neutrophils that invade the brain parenchyma after breach of blood brain barrier with PVD. Since the ephrin receptors are major substrates of MMPs and EphrinB1 ligands are known to be expressed by neutrophils, we therefore determined whether neutrophils expressed ephrins. In Western blots, we confirmed that EphB1 receptors were upregulated in cortical extracts from PVD-injured rat brains. In contrast, EphB1 expression was lower in minocycline-treated rats and was undetectable in sham animals. Using confocal imaging, we found that neutrophils, but not microglia (ED1), expressed EphB1 in the PVD lesion sites. In contrast, both neutrophils and microglia expressed EphB2 receptors and EphrinB2 ligands in the lesion areas. Moreover, EphA4 was also found in neutrophils and microglia after PVD. Surprisingly, we did not observe ephrins and MMPs in astrocytes both inside and outside the lesion sites. Finally, we confirmed that circulating neutrophils expressed ephrins in non-injured rats. Together these results suggest that expression of ephrins and MMPs by neutrophils contributes to lacuna-like cavity formation in ischemic brains.

Disclosures: F.S. Cayabyab: None. **W. Walz:** None. **K. Gowribai:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.10/CC4

Topic: C.09. Ischemia

Title: Neuroprotection of ethyl pyruvate in the postischemic brain by inhibiting HMGB1 release

Authors: *H. LEE[1], J.-H. SHIN[2], I.-D. KIM[1], L. LUO[1], H.-B. LEE[1], J.-K. LEE[1];
[1]anatomy, Inha Univ., Incheon, Korea, Republic of; [2]Seoul Univ., Seoul, Korea, Republic of

Abstract: Ethyl pyruvate (EP), a simple aliphatic ester of pyruvic acid, has been shown to have anti-inflammatory effects and to confer protective effects in various pathological conditions. For example, EP inhibits secretion of high mobility group box 1 (HMGB1), which is known to be released from dying cells and to aggravate inflammatory pathways. In the present study, we investigated whether EP reduces HMGB1 release in ischemic brain of Sprague-Dawley rats and in cultured microglia. In the postischemic brains (60 min MCAO (middle cerebral artery occlusion)), HMGB1 was released extracellularly, generating dual peaks in cerebrospinal fluid (CSF) at around 1 and 7 days after ischemic insult, which were probably generated from damaged neurons and from activated inflammatory cells, respectively. Here, we showed that treatment with EP 30 min post-MCAO (5 mg/kg, i.v.) reduced both peaks. Moreover, delayed EP treatment from 4 days post-MCAO (5 mg/kg, i.v.) reduced accumulation of HMGB1 in CSF at 7

day post-MCAO without significantly ameliorating ischemic brain damage, indicating that EP-mediated suppression of HMGB1 release is a direct effect. EP markedly suppressed the LPS-induced nuclear translocations of protein kinase C alpha and calcium/calmodulin-dependent protein kinase IV, HMGB1 phosphorylation, and subsequent secretion of HMGB1 induced by LPS in BV2 cells, primary microglia, and RAW264.7 cells. Moreover, EP-mediated above-mentioned effects were also independent of cell death or survival. These results indicate that the inhibition of HMGB1 release by EP contributes, at least in part, to its protective effects.

Disclosures: H. Lee: None. **J. Shin:** None. **I. Kim:** None. **L. Luo:** None. **H. Lee:** None. **J. Lee:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.11/CC5

Topic: C.09. Ischemia

Support: 1R21 NS080062-01A1

1PO1 NS37520-08

AI070813

AI023990

CA072074

Title: Meninges modulate post-stroke brain pathology via resident mast cells

Authors: *A. ARAC[1], M. A. GRIMBALDESTON[3], A. R. B. NEPOMUCENO[2], O. OLAYIWOLA[2], M. P. PEREIRA[2], Y. NISHIYAMA[2], H. VOGEL[2], M. TSAI[2], S. J. GALLI[2], T. M. BLISS[2], G. K. STEINBERG[2];
[1]Neurosurg., Stanford Univ., STANFORD, CA; [2]Stanford Univ., Stanford, CA; [3]SA Pathology, Div. of Human Immunology, Ctr. for Cancer Biol., Adelaide, Australia

Abstract: Mast cells (MCs), perivascular cells best known as effector cells involved in the development of inflammatory processes, do not circulate but are resident in virtually all anatomical sites including brain parenchyma and meninges. Brain parenchymal MCs have been reported to exacerbate stroke pathology, however the role of meningeal MCs, if any, in stroke pathology is not known. To address this, we used ‘mast cell knock-in’ mouse models whereby

genetically MC-deficient mice were selectively repaired of their MC deficiency by engraftment of in vitro grown mast cells, either systemically or locally in the meninges.
The WBB6F1-KitW/W-v MC-deficient mouse model was used. 3 groups were tested: wild-type, MC-deficient, and MC-engrafted (after systemic or meningeal engraftment). Mice were subjected to 30 min occlusion of the middle cerebral artery. Brain swelling and infarct size were assessed by T2-weighted MRI and histology. The immune response was quantified by flow cytometry.
MC-deficient mice had less brain swelling at 3d post-stroke, smaller lesions at 3d and 2wk post-stroke, and fewer brain granulocytes at 3d post-stroke than their corresponding wild-type or systemically MC-engrafted groups, implying that MCs exacerbate ischemic injury. Analysis of the central nervous system MC distribution in wild-type and MC-engrafted mice revealed equivalent numbers of MCs in meninges in both groups but almost no MCs in brain parenchyma of MC-engrafted groups. This suggests that meningeal MCs, rather than parenchymal MCs, are key effectors of stroke pathology. To test this, MCs were engrafted locally into the meninges. These meningeal MC-engrafted mice had significantly more brain swelling, larger infarcts, and more brain granulocytes after stroke than MC-deficient mice.
Our results support the conclusion that meningeal MCs can exacerbate stroke pathology. Hence, targeting these cells may be a novel therapeutic strategy for stroke.

Disclosures: A. Arac: None. **M.A. Grimbaldeston:** None. **A.R.B. Nepomuceno:** None. **O. Olayiwola:** None. **M.P. Pereira:** None. **Y. Nishiyama:** None. **H. Vogel:** None. **M. Tsai:** None. **S.J. Galli:** None. **T.M. Bliss:** None. **G.K. Steinberg:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.12/CC6

Topic: C.09. Ischemia

Support: NIH grant NS34179

Title: Inducible nitric oxide synthase in blood borne neutrophils mediates brain damage after transient focal cerebral ischemia

Authors: *L. GARCIA-BONILLA, J. MOORE, G. RACCHUMI, J. ANRATHER, C. IADECOLA;
Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: Nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) contributes to brain damage after cerebral ischemia (J Neurosci, 17:9157, 1997), but the cell types expressing iNOS and mediating tissue damage have not been elucidated. We hypothesized that iNOS present in neutrophil infiltrating the ischemic brain is responsible for tissue injury. To test this hypothesis, $iNOS^{-/-}$ male mice (7 weeks old) were lethally irradiated and transplanted with wild type bone marrow ($BM^{+/+}$) to produce chimeric mice expressing iNOS in peripheral leukocytes (n=10-15 per group). Five to six weeks after transplant, mice were subjected to transient (30 min) MCAO and injury volume was assessed 3 days later in cresyl violet-stained brain sections. Infarcts in $iNOS^{-/-}BM^{+/+}$ mice, in which iNOS is present in peripheral leukocytes but not in brain cells, were larger (43 mm^3, Mean±SE) than those of controls ($iNOS^{-/-}BM^{-/-}$) (24±3 mm^3, $p<0.05$) and not different from those of $iNOS^{+/+}BM^{+/+}$ mice (35±3 mm^3; $p>0.05$). Thus, the data suggest that iNOS positive peripheral leukocytes aggravate ischemic damage in iNOS null mice. Then, we sought to investigate which cell types express iNOS after ischemia in the BM chimeras. To this end, we performed flow cytometry followed by cell sorting. Infiltrating neutrophils and monocytes, as well as microglia and endothelial cells (EC) were purified from brains of $iNOS^{-/-}BM^{+/+}$ and $iNOS^{+/+}BM^{-/-}$ mice 3 days after MCAO. iNOS gene expression was assessed using qRT-PCR. $iNOS^{-/-}BM^{+/+}$ mice showed iNOS mRNA induction in neutrophils ($14x10^{-3}$ copies/cell number) and, to a lesser extent, monocytes ($2x10^{-3}$). As anticipated, iNOS was not detected in EC or microglia. In $iNOS^{+/+}BM^{-/-}$ mice, iNOS was detected in EC ($16x10^{-3}$), but not microglia or infiltrating leukocytes. To provide independent evidence that neutrophils expressing iNOS contribute to tissue damage, adoptive-transfer experiments were performed. Granulocytic cells (Ly6G+) from $iNOS^{+/+}$ or $iNOS^{-/-}$ mice were isolated by negative selection and $5x10^5$ cells were given retro-orbitally to $iNOS^{+/+}$ or $iNOS^{-/-}$ 24 hours after MCAO (n=7 per group). Consistent with the result in chimeric mice, transfer of $iNOS^{+/+}/Ly6G^+$ cells into $iNOS^{-/-}$ mice increased the infarct at 3 days after MCAO (vehicle: 30±4 mm^3; $iNOS^{+/+}/Ly6G^+$ cells: 46±5 mm^3; $p<0.05$) but not when $iNOS^{-/-}/Ly6G^+$ cells were transferred (27±6 mm^3; $p>0.05$ from vehicle). The findings identify iNOS in blood borne leukocytes, particularly neutrophils, as a mediator of tissue damage in the post-ischemic brain and establish iNOS in circulating cells as a therapeutic target for ischemic brain injury.
Supported by NIH grant NS34179

Disclosures: L. Garcia-Bonilla: None. **J. Moore:** None. **G. Racchumi:** None. **J. Anrather:** None. **C. Iadecola:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.13/CC7

Topic: C.09. Ischemia

Support: American Heart Association

Title: microRNA associated with the inflammatory and immune response to stroke in humans may regulate many important targets including MMP9, TLR4, IL1 and IL6

Authors: *G. JICKLING, B. P. ANDER, X. ZHAN, B. STAMOVA, P. VERRO, D. LIU; Neurol., Univ. of California Davis, Sacramento, CA

Abstract: Background: microRNA (miRNA) are important regulators of messenger RNA translation to protein. We have previously shown that miRNA is differentially expressed in the brain and blood of rodents with ischemic stroke compared to controls. In this study we sought to determine whether miRNA is differentially expressed in patients with ischemic stroke, and potentially regulate aspects of the immune response to ischemic brain injury.
Methods: miRNA from 22 patients with ischemic stroke were compared to 22 controls matched for age, sex, race and vascular risk factors. miRNA was isolated from blood, and processed on Affymetrix miRNA 3.0 microarrays. In silico analysis was performed to identify miRNA targets of importance to stroke.
Results: We identified 31 miRNA that were differentially expressed in patients with ischemic stroke compared to controls (corrected p-value <0.05, fold change>|1.2|). These miRNA are predicted to regulate the translation of several molecules important in ischemic stroke, including matrix metalloproteinase 9, toll like receptor 4, interleukin 1 beta, tumor necrosis factor alpha and interleukin 6.
Conclusions: Several miRNA were identified as differentially expressed in the blood of ischemic stroke patients compared to controls. These may have potential importance as markers of brain ischemia, and as regulators of biological functions and pathways involved in the inflammatory and coagulation response in ischemic stroke.

Disclosures: G. Jickling: None. **B.P. Ander:** None. **X. Zhan:** None. **B. Stamova:** None. **D. Liu:** None. **P. Verro:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.14/CC8

Topic: C.09. Ischemia

Support: NHMRC (Australia) Project Grant 1026054

Flinders University Faculty of Health Sciences Research Grant

Title: Peri-infarct responses of microglia and astrocytes in a photothrombotic model of stroke in rats

Authors: *N. R. SIMS, W. YEW, N. D. DJUKIC, J. S. P. JAYASEELAN, H. MUYDERMAN; Discipline of Med. Biochem. Flinders Univ., Adelaide, Australia

Abstract: Infarcts produced by a stroke trigger changes in neighbouring microglia and astrocytes that ultimately lead to formation of a glial scar. Indirect evidence suggests that responses of these glial cells can limit the plastic changes in neurons that are an important contributor to restoration of function following a stroke. Thus, the glial cell responses provide a potential target for treatments that can enhance recovery even when initiated many hours to days after stroke onset. The development of peri-infarct glial cell responses have not been analysed in detail, particularly in the photothrombotic model of stroke that has been widely used to study neuronal plasticity. In the present investigation, photothrombosis was induced in the forelimb motor cortex of male Sprague-Dawley rats. Brains were analysed between 3 h and 7 days later using cresyl violet staining, immunoblots and immunohistochemistry for cell specific markers. Rats showed severe deficits in a forepaw placing test by 3 h that persisted at 3 days and then partially recovered by 7 days. Infarcts identified from cresyl violet staining were readily detectable at 3 h and attained a maximum volume by 24 h. Morphological changes in peri-infarct microglia were seen as early as 3 h. At 24 h, these cells exhibited marked activation as revealed by gross changes in cell morphology and the expression of the marker protein, cyclooxygenase-2. Furthermore, such changes extended well beyond the peri-infarct region. In contrast, astrocytic changes detected from expression of vimentin and glial fibrillary acidic protein, were essentially absent at 24 h. By 7 days, the infarct volume was greatly reduced due to a combination of lesion contraction and invasion of the lesion by microglia and by astrocytic processes. Between 1 and 7 days, the area fraction of immunolabelling for markers of both microglia and astrocytes increased several fold in the peri-infarct tissue, at least in part explained by large increases in cell numbers. These studies provide evidence of earlier and more extensive activation of microglia than has been reported previously following stroke. The microglial changes apparently precede responses in astrocytes by many hours. The studies have also established markers of glial cell changes that will facilitate future investigations into mechanisms underlying treatments that enhance neurological recovery.

Disclosures: N.R. Sims: None. **W. Yew:** None. **N.D. Djukic:** None. **J.S.P. Jayaseelan:** None. **H. Muyderman:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.15/CC9

Topic: C.09. Ischemia

Support: NHMRC Project Grant 1026054

Flinders University Faculty of Health Sciences Project Grant

Title: Early responses of microglia to tissue infarction in a photothrombotic model of stroke in rats

Authors: *W. YEW, J. S. P. JAYASEELAN, N. D. DJUKIC, H. MUYDERMAN, N. R. SIMS; Flinders Univ., Adelaide, Australia

Abstract: Neuronal plasticity in tissue immediately surrounding an infarct is likely to contribute to functional recovery following a stroke. Changes in peri-infarct glial cells influence the extent of neuronal plasticity and therefore are a possible therapeutic target to enhance recovery. Microglia in this tissue are thought to play a critical role in initiating these glial cell responses. However, there have been few attempts to characterise the early responses of these cells or to assess approaches for manipulating the changes. We have addressed these issues in a model of stroke that has been widely used to study neuronal plasticity. Rats were subjected to photothrombotic stroke targeting the forelimb region of the primary motor cortex. At 3 and 24 h after the stroke, forelimb function was assessed (paw-placing test) and the brains perfusion fixed and sectioned for cresyl violet staining and immunohistochemistry for neurons (NeuN) and microglia (Iba1). A measure of circularity of Iba1-positive cells was developed that provided a sensitive indicator of morphological changes indicative of activation. Area fraction of the Iba1 immunoreactivity (percentage of Iba1-positive pixels in thresholded images) was used to detect changes associated with cell activation, migration or cell death. At 3 h after stroke induction, function of the contralateral forelimb was markedly impaired and a lesion readily detectable by cresyl violet staining and NeuN immunolabelling. Microglial circularity was significantly increased in peri-lesional tissue suggesting activation of these cells had been initiated at this time. At 24 h, the impairment of forelimb function persisted and lesion volume (from cresyl violet staining) had increased. Morphological changes in peri-infact microglia were much larger than at 3 h and were also detectable in cortical tissue distant from the lesion. The area fraction of Iba1 in peri-infarct tissue was decreased relative to that in other parts of the ipsilateral cortex. Treatment at 1 h after stroke with minocycline (50 mg / kg i.p.), a widely-used inhibitor of microglial activation, did not significantly alter either the microglial response or forelimb function at 24 h. These results provide evidence for rapid microglial activation that spreads well beyond the peri-infarct tissue. Changes in Iba1 area fraction suggest a subsequent depletion of microglia from the peri-infarct tissue, perhaps due to migration into the lesion or selective cell death that extends beyond the infarct.

Disclosures: W. Yew: None. **J.S.P. Jayaseelan:** None. **N.D. Djukic:** None. **H. Muyderman:** None. **N.R. Sims:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.16/CC10

Topic: C.09. Ischemia

Support: AHA-13BGIA13820011

Title: Lipocalin 2 is a detrimental factor induced after stroke-reperfusion injury

Authors: G. WANG[1], Y.-C. WENG[2], X. HAN[2], *W.-H. CHOU[2,1];
[1]Biomed. Sci., [2]Biol. Sci., Kent State Univ., Kent, OH

Abstract: Stroke is a leading cause of morbidity and mortality worldwide, yet limited therapeutic options exist. The plasma concentration of lipocalin-2 (LCN2) is elevated in human patients with acute ischemic stroke, but its role in stroke is unknown. Recent studies revealed the ability of LCN2 to induce apoptosis. The LCN2 internalized by its cell surface receptor, brain type organic cation transporter (BOCT), chelates intracellular iron and induces apoptosis. Here we show that LCN2 proteins were induced in mouse plasma and ipsilateral hemisphere after transient middle cerebral artery occlusion (tMCAO). The induction of LCN2 proteins in the ischemic hemisphere was detected in the infiltrated neutrophils and a subset of reactive astrocytes. The BOCT displayed a punctate staining pattern in the microtubule-associated protein-2 (MAP-2) positive cultured neurons and brain tissues. To determine the role of LCN2 in vivo, we induced tMCAo in wild-type and LCN2 null mice, and found that the LCN2 null mice showed reduced infarct size and improved neurological outcomes after tMCAo. The recombinant LCN2 proteins when added to the primary cultured neurons caused cell death in a dose-dependent manner. These results indicate that LCN2 is a detrimental factor induced after ischemic stroke, and suggest that reduction of LCN2 could prove useful in the treatment of stroke.

Disclosures: G. Wang: None. **Y. Weng:** None. **W. Chou:** None. **X. Han:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.17/CC11

Topic: C.09. Ischemia

Support: NIH Grant AT006593

Solae, LLC

Illinois Soybean Association

Title: Influences of diet and stroke on expression of genes that modulate inflammation and neuronal remodeling in the adult and aged male rat cerebral cortex

Authors: *E. D. GRISLEY[1], T. REHNBERG[1], J. A. MACLEAN, II[1], W. J. BANZ[3], D. A. BUTTEIGER[4], J. L. CHEATWOOD[1,2];
[1]Physiol., [2]Anat., SIU Sch. of Med., Carbondale, IL; [3]Animal Science, Food, and Nutr., SIUC, Carbondale, IL; [4]Solae, LLC, St. Louis, MO

Abstract: Stroke is a leading cause of lasting neurological deficit in humans, and few options are available for improving outcomes. We have previously shown that rats fed a diet containing soy protein isolate exhibited significantly lower behavioral deficits for 4 weeks following stroke when compared to rats fed a diet containing sodium caseinate. However, the mechanism mediating these observed benefits was not clear. Herein, we describe the effects of diet on several key anti-inflammatory proteins and markers of neuroanatomical remodeling in uninjured rats and at 3 and 7 days after middle cerebral artery occlusion. As before, rats were fed semi-purified diets containing either soy protein isolate or sodium caseinate as the protein source. A third diet group was used in which the soy isoflavones genistein and daidzein were added to the sodium caseinate diet, in an effort to determine the role of these compounds in the previously observed neuroprotection. Rats were pre-fed the appropriate study diet for two weeks prior to stroke. All rats in the Day +3 and Day +7 time points underwent a permanent unilateral middle cerebral artery occlusion, as described previously, and were euthanized on either Day +3 or Day +7, respectively. Brains were removed and tissue from each cerebral hemisphere was isolated and placed in Trizol for mRNA extraction. Relative gene expression was determined for each sample via quantitative real time PCR. Genes of interest in the current study included *Pparg*, *Ywhae* (*14-3-3e*), *Arg1*, *Sod1*, *Gap43*, and *Syp*. At the time of abstract preparation, no significant difference was detected in expression of *Pparg* in any groups of adult male rats. Analyses of additional genes and aged rats are ongoing.

Disclosures: E.D. Grisley: None. **T. Rehnberg:** None. **J.A. MacLean:** None. **W.J. Banz:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Solae, LLC. **D.A. Butteiger:** A. Employment/Salary (full or part-time):; Sola, LLC. **J.L. Cheatwood:**

C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Solae, LLC.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.18/CC12

Topic: C.09. Ischemia

Title: Blood-derived Ly6C^{low} macrophages contribute to spontaneous recovery after stroke

Authors: A. ARAC, Y. NISHIYAMA, T. HIU, T. M. BLISS, *G. K. STEINBERG; Stanford Univ., STANFORD, CA

Abstract: Understanding the mechanisms of post-stroke inflammation is essential to develop therapeutics that would promote recovery. Macrophages are known to promote both injury and repair in various pathologies, and these divergent properties may be due to different macrophage subtypes. Here, we investigated whether the brain macrophage subtypes alter the post-stroke outcome.

C57BL/6 mice (11-14 weeks old) were subjected to transient middle cerebral artery occlusion (tMCAo). Flow cytometry was used to analyze the immune cells in brain, blood. To deplete blood monocytes, clodronate filled liposomes (CL) were used. Phosphate-buffered saline (PBS)-filled liposomes (PL) served as controls. Lesion size was assessed by TTC at 2d post-stroke and silver staining at 7d post-stroke. A time-course analysis of immune cells after stroke identified two macrophage subpopulations in the brain with different temporal profiles: Ly6C^{high} macrophages were the dominant cell population early after stroke, reaching a peak by 3d, and then decreasing in number by 5d. Conversely, the number of Ly6C^{low} macrophages were initially low and peaked by 5d. To investigate the effects of these cells, we used clodronate depletion of monocytes with different injection paradigms. Injecting clodronate 10 min plus 2d after stroke had no significant effect on infarct size at 2d and 7d post-stroke, or on functional deficit, compared to the PL group. However, when the injections were given every other day after stroke, from day 0 to day 6, CL-treated mice had significantly larger infarct sizes at 7d post-stroke (48.9±4.3 vs 35.6±5.8, % of contralateral hemisphere, n=10; $p<0.01$), and worse neurological scores (11.1±1.1 vs 6.2±1.1, n=10; $p<0.01$) than PL-treated mice. Furthermore, this injection paradigm resulted in significantly fewer Ly6C^{low} macrophages in the CL-treated group, suggesting a protective role for these cells.

Macrophage subtypes may have distinct roles in promoting brain damage and recovery after stroke.

Disclosures: A. Arac: None. **Y. Nishiyama:** None. **T. Hiu:** None. **T.M. Bliss:** None. **G.K. Steinberg:** None.

Poster

251. **Ischemia: Inflammation: White Cells and Cytokines**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 251.19/CC13

Topic: C.09. Ischemia

Title: TLR2-induced MMP9 activation compromise blood brain barrier and enhances brain damage in collagenase-induced Intracerebral Hemorrhage

Authors: *S. LEE[1], H. MIN[1], J. HONG[1], Y. JANG[1], K. KWAK[1], S. LEE[2];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Dept. of Microbiology, Sch. of Systems Biol., Chungnam Natl. Univ., Daejeon, Korea, Republic of

Abstract: Innate immune response plays an important role in the pathogenesis of cerebral ischemia/reperfusion injuries. Recent studies have shown that Toll-like receptor 4 (TLR4) is involved in the innate immune responses after cerebral ischemia/reperfusion injuries, yet, the role of TLR2 in stroke or the mechanisms of its function have not been resolved. In this study, we tested it in a collagenase-induced mouse intracerebral hemorrhage (ICH) model using TLR2 knock-out (KO) mice. To induce ICH, collagenase or blood was injected into the right caudate putamen in 8-10 week old male mice. TLR2 expression was upregulated in the ipsilateral hemorrhagic tissues of the collagenase-injected mice. Brain injury volume and neurological deficits following ICH were reduced in the TLR2 KO mice as compared to the wild type (WT) mice. Heterologous blood-transfer experiments show that TLR2 signaling in the brain-resident cells, but not leukocytes, contributes to the injury. In our study to elucidate underlying mechanisms, we found that damage in the blood-brain barrier (BBB) integrity following the ICH was attenuated in the TLR2 KO mice compared to the WT mice, which may be due to reduced MMP-9 activation in brain astrocyte. The reduced BBB damages accompanies with reduced neutrophil infiltration and proinflammatory gene expression the injured brain parenchyma, which may account for the attenuated brain damage in the TLR2 KO mice after ICH. Conclusively, these data demonstrate that TLR2 contributes to brain injury following ICH by compromising BBB through activating MMP-9 in brain.

Disclosures: S. Lee: None. **H. Min:** None. **J. Hong:** None. **Y. Jang:** None. **K. Kwak:** None. **S. Lee:** None.

Poster

252. "Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.01/CC14

Topic: C.10. Demyelinating Disorders

Title: Fingolimod causes antidepressant-like effects and corrects the reduction in hippocampal BDNF levels in mice exposed to chronic unpredictable stress

Authors: *L. DI NUZZO[1], R. ORLANDO[1], J. MIELE[1], P. DI PIETRO[3], M. MOTOLESE[3], A. CARUSO[1], S. SCACCIANOCE[1], G. BATTAGLIA[3], V. BRUNO[1,3], C. POZZILLI[2], F. NICOLETTI[1,3];
[1]Physiol. and Pharmacol., [2]Neurol., Univ. Sapienza, Rome, Italy; [3]Neurosci., I.R.C.C.S. Neuromed, Pozzilli, Italy

Abstract: Recent evidence suggests that fingolimod, the first oral drug approved for the treatment of relapsing-remitting multiple sclerosis, exerts a direct effect on the CNS independently of its peripheral action on the immune system. We and others have found that nanomolar concentrations of fingolimod protect cultured neurons against excitotoxic death through the activation of type-1 sphingosine-1-phosphate (S1P1) receptor (Di Menna et al., Pharmacol Res. 67, 2013; Deogracias et al., Proc Natl Acad Sci USA 109, 2012). Deogracias and collegues also showed that systemic treatment with fingolimod enhances hippocampal levels of brain-derived neurotrophic factor (BDNF). Hippocampal BDNF levels have been associated to the pathophysiology of major depression and to responses to antidepressant medication. Hence, we decided to examine whether fingolimod could cause antidepressant-like effects in the chronic unpredictable stress (CUS) model of "reactive" depression. C57BL/6J adult male mice were exposed to a 4-week CUS protocol, which was sufficient to induce depressive-like behaviour at the forced swimming test (FST). CUS mice showing an increased immobility time at the FST also displayed a substantial reduction in hippocampal BDNF levels. Fingolimod (3 mg/Kg i.p.) was administered daily in the last week of CUS and the treatment was prolonged for 3 weeks after the discontinuation of the stress protocol. Fingolimod reduced the time spent immobile at the FST in about 30% of treated animals, which are considered 'responders' to the drug. In these responder mice, Western blot analysis showed an increase of hippocampal BDNF protein levels, which were restored to values similar to control animals. Fingolimod did not show any effect on BDNF concentrations in mice that did not respond to the drug at the FST. These data show for

the first time an antidepressant-like activity of fingolimod. This raises the interesting possibility that fingolimod may relieve depressive symptoms associated with multiple sclerosis.

Disclosures: L. Di Nuzzo: None. **R. Orlando:** None. **J. Miele:** None. **P. Di Pietro:** None. **M. Motolese:** None. **A. Caruso:** None. **S. Scaccianoce:** None. **G. Battaglia:** None. **V. Bruno:** None. **C. Pozzilli:** None. **F. Nicoletti:** None.

Poster

252. **"Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.02/DD1

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: USDA Intramural

California Walnut Commission

Title: Dietary walnut alters neurochemical and behavioral functions in young and old rats

Authors: *D. F. BIELINSKI, S. POULOSE, D. FISHER, M. MILLER, F. RAMIREZ, B. SHUKITT-HALE;
Neurosci. & Aging Lab., USDA Human Nutr. Res. Ctr, Tufts Univ., BOSTON, MA

Abstract: Diets rich in either polyunsaturated fatty acids or polyphenols have been shown to promote indices of brain functions, primarily with effects on the lipid microenvironment or neuronal signaling. Walnuts are known to contain high amounts of omega fatty acids such as alpha linoleic acid (ALA) and linolenic (LA), as well as neuroactive polyphenols and minerals. Previous studies from our laboratory have shown that feeding diets rich in walnuts protected against age-associated behavioral declines in rats, primarily via enhancing protective signaling, reducing inflammation and preventing accumulation of ubiquitinated inclusion bodies in key regions of the brain. In order to assess whether walnut diets affect age-associated changes in the epigenetic phenomenon, young (3 month) and old (18 month) rats were fed with walnut diets at concentrations of 0%, 6% or 9% w/w for 12 weeks, and subjected to a battery of motor and cognitive tests. Blood serum was collected to test for its exogenous protective effects at the beginning (baseline) and end of the feeding period. Brains were collected to examine global DNA methylation as well as gene-specific DNA methylation patterns in key areas of the brain affecting behavior. The behavioral tests showed significant effects of age. Positive effects of the diet were seen for the wire suspension test, with both 6% and 9% walnut groups performing significantly better than controls. The walnut-fed rats also made fewer errors in the radial arm

water maze. BV2 microglial cells treated with serum from walnut-fed animals and then exposed to the endotoxin lipopolysaccharide (LPS) showed reduced stress-induced inflammatory signals compared to serum from control-fed rats. A correlation of serum effects from respective animals to their behavior is being done along with differential epigenetic patterns. Overall results indicate long-term benefits of a walnut diet rich in both polyphenols and omega fatty acids.

Disclosures: D.F. Bielinski: None. **S. Poulose:** None. **D. Fisher:** None. **M. Miller:** None. **F. Ramirez:** None. **B. Shukitt-Hale:** None.

Poster

252. **"Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.03/DD2

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: USDA Intramural

Cherry Marketing Institute

Title: Tart cherries improve working memory in aged rats

Authors: *B. SHUKITT-HALE, M. G. MILLER, D. F. BIELINSKI, S. M. POULOSE; USDA, ARS, Human Nutrit Res. Ctr. On Aging, BOSTON, MA

Abstract: Aged rats show impaired performance on cognitive tasks that require the use of spatial learning and memory. In previous studies, we have shown the beneficial effects of various dark-colored berry fruits (blueberries, strawberries, and blackberries) in reversing age-related deficits in behavioral and neuronal function when fed to rats from 19-21 months of age. These effects may be due to the phytochemicals in the fruits, which increase antioxidant and anti-inflammatory levels, and directly affect signaling and autophagy in the brain. Tart cherries, like other berry fruits, are rich in polyphenolics, particularly anthocyanins. Thus, the present studies were carried out to determine if tart cherries, added to the diet of 19 mo Fischer 344 rats at 2% for 8 weeks, would be efficacious in reversing the deleterious effects of aging on cognitive behavior as measured with the working memory version of the Morris water maze. Results showed that the cherry diet improved working memory in the Morris water maze, but not in the control group. However, reference memory was better in the control group, possibly because they were not using spatial strategies to solve the maze. Currently, westerns are being done on brain tissue homogenates to measure the levels of four markers involved in the aggregation of polyubiquitinated proteins and activation of autophagy: phospho-mTOR, beclin-1, p62, and

LC3-I/II. We will then be able to assess whether alterations in these autophagy markers may be involved in the mechanisms of action through which the tart cherry polyphenols produce their effects.

Disclosures: B. Shukitt-Hale: None. **M.G. Miller:** None. **D.F. Bielinski:** None. **S.M. Poulose:** None.

Poster

252. **"Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.04/DD3

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: AIBMR Life Sciences Inc.

Title: Dietary acai fruit improves cognition in aged rats

Authors: *M. G. MILLER[1], D. FISHER[2], A. N. CAREY[3], S. M. POULOSE[2], B. SHUKITT-HALE[2];
[1]Psychology, Tufts Univ., Medford, MA; [2]Neurosci. and Aging, USDA HNRC on Aging at Tufts Univ., Boston, MA; [3]Psychology, Simmons Col., Boston, MA

Abstract: Acai is a black-purple fruit (genus Euterpe) cultivated in the Amazon delta and in Brazil (Euterpe oleracea Mart.; EO), as well as Bolivia (Euterpe precatoria Mart.; EP). The fruit's pulp is known to be rich in polyphenolics that may affect cell-to-cell signaling, receptor sensitivity, inflammatory enzyme activity, oxidant/antioxidant balance, and gene regulation. In previous studies, we have shown the beneficial effects of various berry fruits (blueberries, strawberries, and blackberries) in reversing age-related deficits in behavioral and neuronal function when fed to aged rats. Thus, the present study was carried out to determine if EO or EP, fed in the rat diet at 2% for 8 weeks, would be efficacious in reversing the deleterious effects of aging on cognitive behavior in 19-21 mo old Fischer 344 rats. Both the EO and EP diet improved working memory, relative to controls; however, only the EO diet improved reference memory. When BV2 cells were pretreated with serum collected from the rats and then treated with LPS, serum from EO fed rats reduced nitrite release and serum from both acai-fed groups reduced TNF-alpha release. Reductions in nitrite and TNF-alpha release in the cell model were associated with improved water maze performance. Western blots were conducted on BV-2 lysates to measure the levels of two inflammatory markers: inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Treatment of BV-2 cells with serum from acai-supplemented rats

resulted in significant decreases in iNOS and COX-2 expression. Protection of memory during aging by dietary acai may result from its anti-oxidant and anti-inflammatory properties.

Disclosures: M.G. Miller: None. **D. Fisher:** None. **S.M. Poulose:** None. **B. Shukitt-Hale:** None. **A.N. Carey:** None.

Poster

252. "Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.05/DD4

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: Elucidate the neuroprotective mechanisms of Scutellaria lateriflora

Authors: M. LOHANI[1], M. AHUJA[2], M. BUABEID[2], D. BHATTACHARYA[2], A. ROMANDO[3], V. SUPPIRAMANIAM[2], D. SHANNON[4,3], R. AMIN[2], *E. S. COLEMAN[1], D. SCHWARTZ[1], B. KEMPPAINEN[1], M. DHANASEKARAN[2];
[1]Auburn Univ, Coll of Vet Med., Auburn, AL; [2]Harrison school of Pharmacy, Auburn Univ., Auburn, AL; [3]USDA,, Oxford, MS; [4]Col. of Agriculture, Auburn Univ., Auburn, AL

Abstract: Introduction: Scutellaria lateriflora (American skullcap), a native plant of North America, has been used by Americans and Europeans as a nerve tonic and an anxiolytic agents. GABA and glutamate are the major neurotransmitters associated with hyperarousal and excitotoxicity. Bioactive compounds (phytochemicals) present in the medicinal plants affect the neurotransmission and also can have neuroprotective properties. The neuroprotective effects of Scutellaria lateriflora (alcoholic extract) are not fully elucidated. Therefore, the objective of the current study is to investigate the neuroprotective mechanisms of Scutellaria lateriflora.
Methods: Neuroprotective effects were evaluated against hydrogen peroxide induced cytotoxicity using cell viability assay. The antioxidant and anti-apoptotic potential of Scutellaria lateriflora was determined in differentiated hippocampal (H19-7) and pheochromocytoma (PC-12) cells. Furthermore, the effect on glutamatergic receptor expression was also studied. One way ANOVA and appropriate post hoc test was used for finding statistically significant differences between each mean value at $P \leq 0.05$.
Results and Conclusion: Scutellaria lateriflora dose-dependently suppressed caspase-3 expression and scavenged the reactive oxygen species significantly. Decreased reactive oxygen species and caspase activity strongly correlated with the increased cell viability. Additionally, Scutellaria lateriflora increased N-methyl-D-aspartate(NR2A and NR2B) receptors, phosphorylated cAMP response element-binding protein (pCREB) and brain-derived

neurotrophic factor(BDNF) expressions in H19-7 cells. Therefore, the findings of the current research indicate that Scutellaria lateriflora exhibit neuroprotection by affecting the glutamatergic neurotransmission and exhibiting antioxidant and anti-apoptotic action. Thus, Scutellaria lateriflora can be an effective botanical therapy against various neurodegenerative diseases.

Disclosures: M. Lohani: None. **E.S. Coleman:** None. **D. Schwartz:** None. **B. Kemppainen:** None. **R. Amin:** None. **M. Ahuja:** None. **D. Bhattacharya:** None. **M. Buabeid:** None. **M. Dhanasekaran:** None. **V. Suppiramaniam:** None. **D. Shannon:** None. **A. Romando:** None.

Poster

252. **"Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.06/DD5

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSERC

CFI

Title: Chemical analysis and effect of blueberry and lingonberry fruits and leaves against glutamate-mediated excitotoxicity

Authors: *S. KALIDINDI[1], P. VYAS[2], L. CHIBRIKOVA[1], A. U. IGAMBERDIEV[2], J. T. WEBER[1,3];
[1]Sch. of Pharm., [2]Dept. of Biol., [3]Fac. of Med., Mem. Univ. of Newfoundland, St.John's, NL, Canada

Abstract: Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are natural processes occurring in the brain. However, overproduction of ROS and RNS may occur during aging and contribute to neurodegenerative diseases and disorders such as stroke. Phenolic compounds are a large class of phytochemicals that are widespread in the plant kingdom and are known to have antioxidant capacities. This study aimed to determine the radical scavenging capacity and reducing power, as well as the polyphenolic content in fruits and leaves of blueberries and lingonberries growing in Newfoundland. We also determined the potential neuroprotective effect of extracts against glutamate-mediated excitotoxicity, which is believed to contribute to disorders such as stroke and neurodegenerative diseases. We found that extracts of fruits and leaves of blueberry and lingonberry plants had high levels of total soluble phenolics, anthocyanins, tannins, and flavonoids. Overall, the levels of these compounds were significantly

higher in the leaves of these plants versus the fruits. Total antioxidant capacity, in terms of radical scavenging activity and reducing power, were much higher in the leaves of both plants as compared to their fruits. We next tested the effects of the extracts against glutamate-mediated excitotoxicity, a pathological process partially involving overproduction of ROS and RNS. Brain-derived cortical cell cultures from neonatal rat pups were prepared and grown for 9-16 days in vitro. Cells were exposed to glutamate (100 μM) for 24 hours. Glutamate-exposed cells displayed morphological alterations such as disrupted cell bodies, and increased dark punctae, which is often indicative of condensed nuclei. Glutamate also caused a ~23% cell loss after 24 hours as determined by the amount of DAPI-positive nuclei. While lingonberry fruit extract did not provide protection from glutamate toxicity, blueberry fruit extracts were extremely protective. Cultures treated with leaf extracts of lingonberry and blueberry showed no cell loss in the presence of glutamate, indicating a strong protective effect of both the leaf extracts. The overall greater protective effect of leaf extracts was in correlation with the levels of phenolics and antioxidant capacity. These findings suggest that berries or their components may provide protection to the brain from various pathologies. This protective effect of berry extracts may be due to a decrease in oxidative stress, nitrosative stress, or other damaging mechanisms caused by exposure to glutamate.

Disclosures: S. Kalidindi: None. **P. Vyas:** None. **L. Chibrikova:** None. **A.U. Igamberdiev:** None. **J.T. Weber:** None.

Poster

252. **"Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.07/DD6

Topic: D.13. Motor Neurons and Muscle

Title: Vitamin D and/or calcium deficient diets preferentially affect type II muscle fiber neuromuscular junction genomic expression and innervation

Authors: *D. J. GIFONDORWA[1], Y. WANG[1], B. L. ADAMS[2], B. C. YADEN[1], J. G. MACKRELL[1], P. K. SHETLER[1], T. D. THOMPSON[3], K. TYLER[1], K. L. KNOPP[2], Y. L. MA[1], V. KRISHNAN[1], H. U. BRYANT[1];
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Abstract: Low serum vitamin D levels have been associated with reduced physical ability in older adults, resulting in abnormal balance and coordination with an increased incidence of falls. Type II muscles are initially recruited to prevent falls and maintain balance in humans. The exact

mechanism of how vitamin D deficiency produces these deficits remains to be elucidated. The purpose of the current study is to provide insight into how reduced vitamin D levels preferentially affect the neuromuscular junction (NMJ) integrity of type II muscle fibers. Studies with genetically manipulated mice and those with a vitamin D modified diet have provided valuable insights which have translated into humans. Previous work has demonstrated that mice fed a vitamin D deficient diet at the time of weaning and continuing for six weeks is sufficient to increase the total body muscle/fat mass ratio, while decreasing performance on locomotor behavioral tasks and NMJ innervation of type II skeletal muscles. The heat stress response was also determined to be more highly activated in type I compared to type II skeletal muscle, as determined by increases in mRNA and protein expression of heat shock proteins 70, 90 and the DnaJ homologue cysteine string protein.
The current study was designed to investigate the role of dietary vitamin D and/or calcium in regulating muscle function in a heterogeneously fiber comprised skeletal muscle. Three week old mice were fed diets with varied combinations of vitamin D and calcium. Serum levels of calcium and parathyroid hormone (PTH) along with behavioral testing were performed to confirm previous results. As expected, body composition and behavioral data from animals fed with a vitamin D deficient diet correlated with previous studies. Gastrocnemius force generation was performed and as expected the VD^-/Ca^- group had decreased measures including but not limited to maximum tetanic
force and twitch tension as compared to the other three dietary groups that contained vitamin D and/or calcium. Preliminary findings from gait analysis suggest decreased stride length in both the forelimbs and hindlimbs of mice fed a vitamin D deficient diet. Genomic and single fiber analysis, along with histology will be done to assess multiple aspects of neuromuscular junction integrity and fiber type composition in the gastrocnemius. The results of the study will provide insight into how a vitamin D and/or calcium deficient diet detrimentally affects mobility in the context of alterations in specific muscle fiber types, within a mixed fiber type skeletal muscle, resulting in changes that regulate structure and function of the NMJ.

Disclosures: D.J. Gifondorwa: None. **Y. Wang:** None. **B.L. Adams:** None. **B.C. Yaden:** None. **J.G. MacKrell:** None. **P.K. Shetler:** None. **T.D. Thompson:** None. **K. Tyler:** None. **K.L. Knopp:** None. **Y.L. Ma:** None. **V. Krishnan:** None. **H.U. Bryant:** None.

Poster

252. **"Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"**

Location: Halls B-H

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Program#/Poster#: 252.08/DD7

Topic: D.13. Motor Neurons and Muscle

Support: NIH grant 1 R15 AG022908-01A2

NSF grant DBI 0552517

Western Michigan University

Title: Walk-training increases expression of glial cell line-derived neurotrophic factor in pectoralis muscle but not diaphragm from mouse

Authors: *E. K. DONOVAN[1], M. J. MCCULLOUGH[2], J. M. SPITSBERGEN[2];
[2]Biol. Sci., [1]Western Michigan Univ., Kalamazoo, MI

Abstract: Neurotrophic factors are proteins that play an important role in the development and maintenance of neurons. Recent studies have shown that neurotrophic factors may hold promise for treating damage to the nervous system caused by trauma or diseases, such as amyotrophic lateral sclerosis or Parkinson's disease. Glial cell line-derived neurotrophic factor (GDNF) is expressed in skeletal muscles and affects peripheral motor neurons. Results of previous studies have demonstrated that exercise can increase GDNF content in skeletal muscle of rat. The goal of the current study was to examine whether expression of GDNF in skeletal muscle of mouse is also regulated by physical activity. Our hypothesis states that muscles undergoing higher levels of contractile activity will express GDNF at higher levels than muscle exhibiting lower levels of contractile activity. For these studies we examined GDNF protein content in tissues from sedentary control mice and in mice following exercise. Muscles examined included the diaphragm (DIA), an involuntary slow twitch muscle and pectoralis major (PEC), a voluntary slow twitch muscle. Treatment groups included 7 control mice and 6 mice that underwent walk training. The treatment group had a one-week training period, and then exercised for 30 minutes a day, at 8 meters/minute, five days a week. Following completion of the exercise regimen muscles were removed and processed for determination of GDNF protein content using enzyme-linked immunosorbant assay, GDNF protein localization and end plate morphology. The results show that DIA from control animals contains more GDNF than PEC, while exercise increased GDNF protein content in PEC but not DIA. These findings suggest that GDNF production is altered by exercise in voluntary slow-twitch muscles, but not involuntary slow-twitch muscles. These results contribute to understanding the mechanisms underlying the normal control of GDNF expression in skeletal muscles. It is important to develop a more complete understanding of normal expression of GDNF in skeletal muscle before we can determine whether GDNF-based therapies may be used in the treatment of traumatized or diseased neural tissues.

Disclosures: E.K. Donovan: None. **M.J. McCullough:** None. **J.M. Spitsbergen:** None.

Poster

252. **"Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.09/DD8

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Support: RO1 DK47320

G12 MD007601

G12 RR003061

Title: Deletion of selenoprotein m leads to obesity without cognitive deficits

Authors: *M. W. PITTS, M. A. REEVES, A. H. HASHIMOTO, A. OGAWA, P. KREMER, L. A. SEALE, M. J. BERRY;
Dept. of Cell and Mol. Biol., Univ. Hawaii, HONOLULU, HI

Abstract: Selenium is an essential trace element that is co-translationally incorporated into selenoproteins in the form of the 21st amino acid, selenocysteine. This class of proteins largely functions in oxidation-reduction reactions and is critically involved in maintaining proper redox balance essential to health. Selenoprotein M (SelM) is a thioredoxin-like ER-resident protein that is highly expressed in the brain and possesses neuroprotective properties. In this study, we first assessed the regional pattern of SelM expression in the mouse brain to provide insights into the potential functional implications of this protein in physiology and behavior. Next, we generated transgenic mice with a targeted deletion of the SelM gene and subjected them to a battery of neurobehavioral tests to evaluate motor coordination, locomotion, and cognitive function in comparison to wild-type controls. Finally, these mice were tested for several measures of metabolic function and body composition. Our results show that SelM knockout mice display no deficits in measures of motor coordination and cognitive function, but exhibit elevated weight gain and increased white adipose tissue deposition. These findings suggest that SelM plays an important role in the regulation of body weight and energy metabolism.

Disclosures: M.W. Pitts: None. **M.A. Reeves:** None. **A.H. Hashimoto:** None. **A. Ogawa:** None. **P. Kremer:** None. **L.A. Seale:** None. **M.J. Berry:** None.

Poster

252. **"Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.10/DD9

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: The effects of proton radiation on neurogenesis in adult mice that have consumed pomegranate juice

Authors: *M. S. DULCICH[1], V. POP[3], N. MISTRY[1], J. BADAUT[2], R. E. HARTMAN[1]; [1]Psychology, [2]Basic Sci., Loma Linda Univ., LOMA LINDA, CA; [3]Univ. of California, Irvine, Irvine, CA

Abstract: Exposure to ionizing radiation may have deleterious effects on physical and mental health, with an increased risk of proton radiation for astronauts traveling outside Earth's atmosphere into lower earth orbit. In animal models, radiation has been shown to suppress neurogenesis in the subgranular zone of the hippocampus, a key area for learning and memory (Rola et al., 2004). In animal and human studies, several antioxidant polyphenols were shown to be neuroprotective (reviewed by Pandey & Rizvi, 2009). Few studies have looked at the effects of proton radiation on the central nervous system, although proton radiation is the most prevalent ionizing radiation in space. This study determined whether the suppression of neurogenesis using proton irradiation in mice could be reversed by ingestion of pomegranate juice, which has high levels of antioxidant and anti-inflammatory polyphenols. Adult C57BL/6 mice received dilute pomegranate juice (n=48) or a control sugar water (n=48) in their drinking bottles for 1 week before irradiation with protons (2 Gy at 150 MeV/n at 1-2 Gy/min) or sham treatment. Sham animals were placed in the same chambers as irradiated mice for the same amount of time, but did not receive radiation beam. Animals were maintained on the pomegranate or control juice for a total of 10 weeks. Subsequent behavioral testing revealed that proton irradiation induced depression-like behaviors, but this effect was ameliorated by the pomegranate diet (Dulcich & Hartman 2013). One day after BrDU injections (50mg/kg body weight, 2x/day for 3 days) were completed, brain tissue was prepared (at 11 weeks post-radiation) for quantification of BrdU+ and DCX+ cells in the dentate gyrus of the hippocampus. Radiation suppressed overall cell proliferation and neurogenesis. Pomegranate juice increased cell proliferation and neurogenesis, but only in non-irradiated animals. Our results demonstrate that proton radiation can suppress neurogenesis in the hippocampus, and that pomegranate juice may be beneficial in promoting neurogenesis.

Disclosures: M.S. Dulcich: None. **V. Pop:** None. **N. Mistry:** None. **J. Badaut:** None. **R.E. Hartman:** None.

Poster

252. **"Neuroprotective Mechanisms: Drugs, Nutrition, and Diet"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 252.11/DD10

Topic: C.12. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effects of aluminum on neurogenesis in subgranular zone of mouse dentate gyrus in high fat diet with D-galactose induced model

Authors: *J. KIM[1], S. NAM[1], D. YOO[1], W. KIM[1], H. JUNG[1], J. CHOI[2], M.-H. WON[3], I. HWANG[1], J. SEONG[1], Y. YOON[1];
[1]Vet. Anat. and Cell Biol., Seoul Natl. Univ. / Col. of Vet. Med., Gwanak-Gu Seoul, Korea, Republic of; [2]Dept. of Anat., [3]Dept. of Neurobio., Kangwon Natl. Univ., Chuncheon, Korea, Republic of

Abstract: Aluminum is the most plentiful metal in earth crust and its usage in cooking utensils, cosmetics, drinking containers, pharmaceutical products, building materials makes many chances of aluminum consumption. However, its toxicity is low and higher amount of deposition is required for the development of its harmful effects. In this study we investigated the effects of aluminum treatment (40 mg/kg/day) on cell proliferation and neuroblast differentiation in the D-galactose-induced aging mice. Additionally, we compared the complex effects of aluminum, D-galactose, and high fat diet on adult hippocampal neurogenesis via immunohistochemistry using the marker protein, Ki67 (a marker for cell proliferation) and doublecortin (DCX, a marker for differentiating neuroblasts). Solely D-galactose, aluminum or high fat diet treatment decreased the level of adult neurogenesis, but the dual treatment exacerbated the reduction of Ki67 and DCX expression. Additionally, aluminum treatment exacerbated the degree of reduction in D-galactose or/and high fat diet treated mice. These results suggest that aluminum decreases the cell proliferation and neuroblast differentiation in the augmentative way.

Disclosures: J. Kim: None. **S. Nam:** None. **D. Yoo:** None. **W. Kim:** None. **H. Jung:** None. **I. Hwang:** None. **J. Seong:** None. **Y. Yoon:** None. **J. Choi:** None. **M. Won:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.01/DD11

Topic: F.01. Human Cognition and Behavior

Title: Brain activation informs risk in genome-wide association

Authors: *B. E. SPENCER[1], R. E. STRAUB[2], Y. TONG[1], K. F. BERMAN[1], D. R. WEINBERGER[2], J. H. CALLICOTT[1];
[1]Clin. Brain Disorders Branch, DIRP, NIMH, NIH, Bethesda, MD; [2]Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: Background: It has been difficult to identify risk genes for complex psychiatric disorders such as schizophrenia, although findings have begun to replicate across very large samples. Alternatively, genetic risk can be studied using intermediate phenotypes, of which prefrontal inefficiency is one (Callicott et al., 2003), under the assumption that some of the genes underlying brain function in health (but abnormal in psychiatric illness) may confer increased risk of disease and with a smaller sample size. As opposed to data-driven GWAS, most intermediate or endophenotype studies have followed the candidate gene approach, although recent evidence suggests these phenotypes can be assayed genome-wide, though no true GWAS-positive findings have emerged (Potkin et al., 2009; Potkin et al., 2008). Here we used imaging data to query genome-wide variants in order to find single nucleotide polymorphisms (SNPs) associated with BOLD fMRI activity during working memory (WM) in healthy people.
Methods: 442 healthy volunteers and unaffected siblings of patients with schizophrenia performed the N-back working memory task on a 3T MRI scanner. Mean values of activation were extracted for each individual's 2Back vs. 0Back contrast map over 22 ROIs defined by the Automated Anatomical Labeling (AAL) atlas. The effects of age and sex were removed from these extracted values in SPSS and residuals were used in this GWAS. These values were tested over 687,947 variants for the 442 subjects using a linear regression in PLINK. Significant effects were then examined using a multiple regression in Statistical Parametric Mapping (SPM5) with age and gender as covariates.
Results: Rs6065270, a SNP upstream of the MAFB gene, was found to significantly affect activation ($p=1.02\cdot10^{-8}$) during the N-back working memory task using the GWAS approach. In parallel, a significant cluster of activation was found in the dorsolateral prefrontal cortex (Brodmann Area 9) ($p<0.01$ FWE whole-brain corrected) for this SNP with minor allele homozygotes displaying prefrontal inefficiency during the N-back task.
Conclusions: In healthy volunteers, rs6065270 predicted changes in prefrontal activation at a genome-wide level and implies imaging data can be used to inform risk SNPs associated with intermediate phenotypes in schizophrenia.

Disclosures: B.E. Spencer: None. **R.E. Straub:** None. **Y. Tong:** None. **K.F. Berman:** None. **D.R. Weinberger:** None. **J.H. Callicott:** None.

Poster

253. Schizophrenia: Altered Brain Network Function

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.02/DD12

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: CIHR

NSERC

Vanier Canada Graduate Scholarships

Title: Are individuals with schizophrenia who experience semantic encoding memory deficits can improve their performance following specific training?

Authors: *S. GUIMOND, M. LEPAGE;
McGill Univ., Montréal, QC, Canada

Abstract: Cognitive deficits appear to be a core characteristic of schizophrenia and there is a great need to develop efficient techniques in order to improve cognitive function in this population. Considering the significant variability of memory difficulties between individuals with schizophrenia, it could be beneficial to develop training which focuses precisely on one specific deficit. A relation between the impairment in associative episodic memory (EM) and the difficulty to self–initiate semantic encoding strategies in people with schizophrenia has been recently proposed. Prefrontal cortex (PFC) dysfunction observed in these patients might also play a role in the difficulty to self-initiate effective semantic encoding strategies in EM. The goal of this study was to design an EM task allowing us to select patients that seem to have this specific deficit and to develop a memory training that would specifically target this problem. Two specific measures of memory were taken: the CVLT (a standardized measure of the use of semantic encoding strategy) and the BVMT (a control spatial memory task). We developed an EM task, in which self-initiation of semantic encoding strategies was isolated in one condition. This memory task was used to select patients with the deficit. Our preliminary data, based on 8 individuals with enduring schizophrenia, revealed that this specific memory deficit seems to be present in approximately half of them. These individuals were selected for two sessions of semantic encoding strategy training, after which they were administered the same task but with new items to memorize, and once again performed the CVLT and the BVMT.
Our preliminary results suggest that the performance of individuals with schizophrenia involved in training improved in the self-initiation condition (Cohen's d= 3.22). Participants also considerably increased the number of semantic clusters for the trials 1-5 of the CVLT (Cohen's d= 3.36), while no clear differences were found on the BVMT.
Our results suggest that half of individuals with enduring schizophrenia seem to experience deficits in semantic encoding strategy in EM. We also developed a memory training program that successfully helped these patients improve their self-initiation of semantic encoding strategy, as demonstrated by their increased performance in the task, and also by the number of semantic clusters used after the training in the CVLT. This study is a part of a larger research project that

will investigate the role of the PFC in the self-initiation of semantic encoding strategy using functional neuroimaging. Therefore, we will further evaluate the impact of the training on prefrontal neural activity.

Disclosures: S. Guimond: None. **M. Lepage:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

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Program#/Poster#: 253.03/DD13

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: HSØ 2012047

Title: Altered functional brain networks in schizophrenia - a resting state fMRI study

Authors: *K. C. SKATUN[1,2], L. T. WESTLYE[1,3], G. P. BIELE[3], O. A. ANDREASSEN[1,2]; [1]Div. of Mental Hlth. and Addiction, KG Jebsen Ctr. For Psychosis Res. - TOP, Oslo Univ. Hosp., Oslo, Norway; [2]Inst. of Clin. Med., [3]Dept. of Psychology, Univ. of Oslo, Oslo, Norway

Abstract: Schizophrenia (SZ) is a severe mental disorder characterized by symptoms such as hallucinations, delusions, and cognitive dysfunction. Magnetic resonance imaging (MRI) studies have documented abnormalities in brain structure and function, with the emerging notion that symptoms in schizophrenia are not only modulated by focal pathologies, but rather aberrant connections between brain regions at the network level. Resting-state functional MRI (rFMRI) targets large-scale functional connectivity patterns in the brain. Previous studies have yielded an inconclusive pattern of spatiotemporal differences in resting-state networks (RSNs) in SZ. The discrepancies may be largely due to methodological differences and relatively small sample sizes. Based on rFMRI we characterized and compared RSNs and their interactions between patients with SZ (n=45) and healthy controls (n=95). Independent component analysis was used to identify brain networks, including the default mode network and fronto-parietal networks. Individual spatial maps and corresponding time series were estimated using dual regression (1, 2). The spatial maps, representing the nodes in the network structure, were compared between groups using permutation testing, and associated time series were submitted to hierarchical clustering based on the temporal correlations. Next, each element in the correlation matrices, representing edges in the network, was submitted to between group comparisons. The results revealed altered functional connectivity between specific brain nodes in SZ, supporting that large-scale brain network configuration during rest show promise as a candidate imaging intermediate phenotype in psychiatry. A detailed characterization of the brain functional

abnormalities in SZ may advance and refine diagnostics, clinical care, and treatment strategies.
1.C. F. Beckmann, C. Mackay, N. Filippini, S. M. Smith, Group comparison of resting-state FMRI data using multi-subject ICA and dual regression. OHBM, (2009).
2.N. Filippini et al., Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci U S A 106, 7209 (Apr 28, 2009).

Disclosures: K.C. Skatun: None. **G.P. Biele:** None. **L.T. Westlye:** None. **O.A. Andreassen:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.04/DD14

Topic: C.16. Schizophrenia and Bi-polar Disorder

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NIH R25 MH060482;

Title: Emotional distractors result in aberrant activation within fronto-polar cortex in schizophrenia

Authors: *D. RAMANATHAN[1], J. M. FORD[1,2], V. D. CALHOUN[3], J. TURNER[3], J. T. VOYDOVIC[4], A. BELGER[5], T. G. M. VAN ERP[6], J. G. VAIDYA[7], D. H. MATHALON[1,2]; [1]Univ. California, San Francisco, San Francisco, CA; [2]Mental Hlth. Service, San Francisco VA Med. Ctr., San Francisco, CA; [3]Mind Res. Network, Albuquerque, NM; [4]Brain Imaging and Analysis Ctr., Duke Univ., Durham, NC; [5]Psychiatry and Psychology, Univ. of North Carolina, Chapel Hill, NC; [6]Dept. of Psychiatry and Human Behavior, Univ. California, Irvine, Irvine, CA; [7]Psychiatry, Univ. of Iowa, Iowa City, CA

Abstract: Subjects with schizophrenia demonstrate differences in the neural processing of emotional stimuli. It has not been entirely clear how these differences in neural processing relate to the disease process or to other aspects of cognition. Prior studies on emotional interference of cognition in schizophrenia have not consistently demonstrated either greater behavioral deficits or greater interference within executive control/prefrontal circuits compared to healthy control individuals. However, prior studies have generally utilized smaller sample sizes with limited variation in task difficulty, both issues that might have confounded results. In this study, through

a large, multi-site collaboration (the Functional Bio-Informatics Research Network, or FBIRN), we were able to recruit a significantly greater sample size than has been previously been utilized in emotion-cognition studies of schizophrenia (a total of 165 schizophrenic subjects and 176 healthy controls). These subjects underwent functional neuroimaging while participating on a Sternberg delayed match to sample working memory paradigm, with either emotional or neutral distracting images displayed during the delay period of the task. Task difficulty was systematically normed for all subjects prior to neuroimaging by individually varying working memory load until accuracy was close to 80%. During the neuroimaging task, each subject was then tested at 3 task-loads (their behaviorally normed load; and loads both lower and higher than that). Using this paradigm, we found that while both patients and healthy control subjects demonstrated emotional interference at high working memory loads ($p<0.0001$ for Emotion; $p<0.001$ Load x Emotion effects) no group differences in the degree of emotional interference were identified. However, emotional distractors during the WM paradigm did result in significantly greater activation within dorsal posterior cingulate and left fronto-polar cortex during low WM loads ($p<0.025$, cluster-corrected for multiple comparisons). Interestingly, the aberrant activation of left fronto-polar cortex following emotional vs. neutral distractors strongly correlated with PANSS scores in the positive ($R = 0.25$, $p <.0001$); negative ($R = 0.15$;$p <0.05$) and general ($R = 0.3$; $p <0.0001$) psychopathology domains. Fronto-polar cortex is often thought of as the apex of executive control and decision-making, particularly suited for long-range planning and goals. These findings suggest that deficits in long-range planning and goals, a feature of schizophrenia, may be related to aberrant function or perturbation of this system by emotional stimuli.

Disclosures: D. Ramanathan: None. **J.M. Ford:** None. **V.D. Calhoun:** None. **J.T. Voydovic:** None. **J. Turner:** None. **A. Belger:** None. **T.G.M. van Erp:** None. **J.G. Vaidya:** None. **D.H. Mathalon:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.05/DD15

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: Elizabeth Elser Doolittle Investigatorship from NARSAD

Joe Young Sr. Fund

Title: Network dysfunction during associative learning in schizophrenia: Direct evidence of disordered cortico-striatal-hippocampal interactions using fMRI and Dynamic Causal Modeling

Authors: *S. WADEHRA[1], P. PRUITT[2], V. DIWADKAR[1];
[1]Wayne State Univ., Detroit, MI; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Introduction: Dynamic Causal Modeling (DCM; Stephan et al., 2010) provides a substantive new approach for investigating disordered network interactions in the schizophrenia brain. Using information priors, DCM permits evaluation of competing models of network architecture distinguished at the second-level using Bayesian selection. Coupling estimates between regions evidence effective connectivity (Friston, 2005) related to endogenous connections and modulatory effects of task on these connections. Here, we applied DCM to assess fronto-hippocampal-striatal interactions in SCZ and controls during paired-associate learning (Diwadkar et al., 2008).
Methods: fMRI (4.0T) was collected in 19 subjects (SCZ=9, controls=10; 18≤age≤35yrs). Because DCM relies on Bayesian model selection (BMS) to identify the most appropriate generative model for the data relative to neurobiologically-plausible competitors, 144 models were constructed by permuting connections between 6 brain regions. In addition to the three primary regions, the supra-network included visual, inferior-temporal, and superior-parietal cortices. These 2,736 models (144 modelsx19 subjects) were submitted to second-level RFX analyses for BMS. Inter-group inferences were based on Bayesian averages of estimated coupling (Penny et al., 2010).
Results: BMS identified one winning model (exceedance probability: 60% greater than its closest competitor). Model architecture revealed reduced fronto-hippocampal coupling, but increased striatal-hippocampal coupling in SCZ.
Conclusion: These preliminary data demonstrate that DCM is sensitive to identifying reduced fronto-hippocampal coupling and compensatory increases in fronto-striatal coupling during associative-learning in schizophrenia. Application of DCM to in vivo fMRI data constitutes a substantive advance in the ability of fMRI to identify mechanisms (rather than mere correlates) of schizophrenia-related pathophysiology (Diwadkar, Wadehra et al., 2012, Arch Gen Psychiatry).

Disclosures: S. Wadehra: None. **P. Pruitt:** None. **V. Diwadkar:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

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Program#/Poster#: 253.06/DD16

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: USPHS awards DA026451

P40RR018604 (Virus Center Grant)

Title: Trans-synaptic retrograde tracing of an auditory cortical circuit from posterior caudatoputamen in the rat

Authors: *A. PARGA[1,3], V. LAVARNWAY[3], E. M. NIKULINA[2], R. P. HAMMER, Jr.[2];
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Abstract: Auditory hallucinations constitute a hallmark in the diagnosis of schizophrenia. Functional studies have reported activation of the auditory cortex (AudCx) during hallucinations, as well as increased dopamine (DA) transmission in the caudate nucleus. In addition, current treatments with classical antipsychotic drugs diminish hallucinations by DA antagonism.
We have shown that DA infusion into the posterior caudatoputamen (CPu) of the rat dose-dependently activates AudCx, as does a sound stimulus, and that co-infusion of selective D_1- and D_2-like antagonists attenuate this effect. The present study sought to describe the neuronal circuit by which manipulation of striatal DA transmission alters functional activity of AudCx. We hypothesized that the posterior CPu affects the primary AudCx through a multi-synaptic striato-pallido-thalamo-cortical (SPTC) circuit. The circuit was traced using recombinant pseudorabies virus (PRV-152), a retrograde transsynaptic tracer that express enhanced green fluorescent protein (eGFP) as an indicator of viral passage between connecting neurons. We infused PRV-152 (100 nl of 7×10^4 pfu/ml) in the primary auditory cortex (coordinates: AP -4.3 mm, ML 6.69 mm, DV -4.45 mm) of adult Sprague Dawley rats. Maps of eGFP-expressing neurons were produced using Neurolucida software (MBF Bioscience; Williston, VT) by tracing the spread of PRV-152 at 24-72 h post-inoculation. Image analysis revealed the spread of PRV-152 across layers within the ipsilateral AudCx at 24 h; subsequently at 72 h, labeling was observed in the ipsilateral ventrolateral nucleus of the thalamus and the posterior CPu bilaterally. Analysis of intermediate post-inoculation times is ongoing. These neuroanatomical data delineate the circuitry which may underlie striatal DA-induced functional activation of AudCx. Further analyses will examine the involvement of both direct and indirect striatal projection neurons, as well as auditory sensory pathways. We propose that this auditory SPTC circuit may represent the biological substrate for intrinsic activation of the AudCx, and the pathophysiology of auditory hallucinations.

Disclosures: A. Parga: None. **V. Lavarnway:** None. **E.M. Nikulina:** None. **R.P. Hammer:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.07/DD17

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Reward processing in unaffected siblings of schizophrenia patients: An fMRI study

Authors: *M. DE LEEUW, R. S. KAHN, M. VINK;
Psychiatry, Rudolf Magnus Inst. of Neuroscience, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract: Schizophrenia is characterized by impaired functioning of the frontal-striatal network: hyperdopaminergic activation in the striatum and hypodopaminergic activation in the frontal cortex. These network dysfunctions result in cognitive deficits such as impaired reward processing. Reward processing can be dived into two sub-processes: anticipation and the outcome of reward. Functional MRI studies in patients have shown hypo-activation of the ventral striatum during reward anticipation. Whether this impaired reward processing is related to the genetic risk of schizophrenia is not known. To answer this question, we investigated reward processing using fMRI in siblings of schizophrenia patients who share on average 50% of their ill siblings' genes. Twenty-eight unaffected siblings and 29 matched controls performed a monetary delayed incentive task during fMRI scanning. All subjects were rewarded in 50% of the reward trials. Despite this equal performance, during reward anticipation siblings showed hypoactivation in the ventral striatum compared with controls. During the outcome of reward, hyperactivation in the ventral striatum and in the orbital frontal cortex was found in siblings compared to controls. These findings are consistent with the notion of impaired dopaminergic functioning in the fronto-striatal network typically associated with schizophrenia. Impaired reward processing may constitute a vulnerability factor for schizophrenia. Twin studies should clarify whether these phenotypic abnormalities are a full genetic risk factor of schizophrenia.

Disclosures: M. De Leeuw: None. **R.S. Kahn:** None. **M. Vink:** None.

Poster

253. Schizophrenia: Altered Brain Network Function

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.08/DD18

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: Zawia University, Libya

Title: Early antipsychotics response in first-episode male schizophrenia patients coincides with rapid modulation of cognitive behaviors and neuronal plasticity in adult male rats

Authors: *M. M. KHAN[1,3], N. T. HWISA[2], A. R. ELTUMI[4], A. E. MEHEMED[1], S. P. MAHADIK[5];
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Abstract: Early response to antipsychotic drugs has been shown to predict subsequent long-term improvement in the first-episode schizophrenia patients. However whether early antipsychotics response in first-episode schizophrenia patients coincides with early antipsychotics effects in animals has not been investigated. In this study we have made a retrospective analysis of the early response of haloperidol, risperidone and clozapine therapy in first-episode male schizophrenia patients (N=108) admitted during the year 2003-2010 to the Al-Razi Mental Hospital, Hay Al Andalous, Tripoli, Libya. Parallel studies were performed to see the early effects of the above antipsychotic drugs on the cognitive behaviour, neurogenesis and synaptic plasticity in normal and pre-trained latent adult male rats. In first-episode schizophrenia patients, early response to haloperidol and risperidone was observed in 68% patients with 51% reduction in the PANSS (Positive and Negative Symptoms Scale) total score at week 1-2. In clozapine treated patients similar early response was found in 48% patients at weeks 2-3. In animal studies, we analysed early effects of antipsychotic drugs in the normal rats as well as rats pre-trained for 10 days on various behaviour paradigms followed by a 2 months latent period, as well as in pre-trained latent rats treated with MK-801. Both haloperidol and risperidone treated animals performed better on various behaviour paradigms after 3-4 days treatment compared to control group. This early-improved performance was associated with a rapid enhancement in the neurogenesis in SVZ, and spine density in the prefrontal cortex. Clozapine treated animals were slow in responding to improvement on various behaviour parameters, as well as induction of neurogenesis and spine density. These results suggest that antipsychotics-induced early response in first-episode male schizophrenia patients coincides with rapid enhancement in the cognitive parameters, neurogenesis and synaptic plasticity in both the normal and pre-trained MK-801 treated animals, however; variations may exist with respect to antipsychotic drugs and their early response both in schizophrenia patients and animals.

Disclosures: M.M. Khan: None. **N.T. Hwisa:** None. **A.R. Eltumi:** None. **S.P. Mahadik:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Janssen Pharmaceutica Research Foundation, US. **A.E. Mehemed:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.09/EE1

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: Wellcome Trust

Title: Common and distinct neural effects of risperidone and olanzapine on procedural learning in schizophrenia

Authors: *V. KUMARI[1], U. ETTINGER[2], S. LEE[3], C. DEUSCHL[3], A. P. P. ANILKUMAR[4], S. C. WILLIAMS[3];
[1]Psychol, Inst. of Psychiatry, London, United Kingdom; [2]Univ. of Bonn, Bonn, Germany; [3]Inst. of Psychiatry, King's Col. London, London, United Kingdom; [4]South London and Maudsley NHS Fndn. Trust, London, United Kingdom

Abstract: Available antipsychotic treatments do not improve all symptoms of schizophrenia. Most cognitive domains show only minimal improvement following typical or atypical antipsychotic treatments, and some may even worsen following typical antipsychotic treatment. One such domain is procedural learning (PL), or sequence learning, a function relying on the integrity of the fronto-striatal system. In this study we ascertained, using functional magnetic resonance imaging (fMRI), whether switching to atypical antipsychotics would normalise PL and task-related activation in patients previously on stable doses of typical antipsychotics. Furthermore, we explored differential effects of the atypical antipsychotics risperidone and olanzapine. The study involved 30 patients with schizophrenia who underwent fMRI on two occasions, at baseline and six weeks later, during a 5-min PL task on which performance is known to improve within a session as a function of practice without the need for conscious awareness. Ten of these 30 patients remained on typical antipsychotics throughout the study while 20 patients were switched randomly in equal numbers to receive either olanzapine or risperidone for 6 weeks. We found that, at baseline, patients (all on typical antipsychotics) showed no PL. At follow-up, those who remained on treatment with typical antipsychotics continued to show lack of PL whereas patients who were switched to atypical antipsychotics displayed significant PL (i.e. improvement) and increased activation in the superior frontal gyrus extending to the inferior frontal gyrus, and the anterior cingulate extending to the striatum. These neural effects were strongly present as a linear increase over five successive 30-s blocks of sequenced trials. A switch to either risperidone or olanzapine resulted in comparable improvement in PL but with partially overlapping and distinct task-related activation. We conclude that atypical antipsychotics are effective in restoring PL deficits and associated neural activity in schizophrenia. Furthermore, different atypical antipsychotics produce idiosyncratic

task-related neural activations and this specificity may contribute to their differential clinical profiles in the longer term.

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Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.10/EE2-DP4

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIMH R01MH85639

Title: Multivariate analysis reveals sex differences in distributed neural networks of attention, language and emotion regulation in typically developing youth and pediatric mania

Authors: *L. IORDANESCU[1], M. C. STEVENS[2], J. FITZGERALD[1], M. N. PAVULURI[1]; [1]Psychiatry, Pediatric Brain Res. And Intervention Center, Univ. of Illinois At Chic, Chicago, IL; [2]Psychiatry, Yale Univ. Sch. of Medicine, Olin Neuropsychiatry Res. Center, The Inst. of Living/Hartford Hosp., Hartford, CT

Abstract: An emerging area of interest in mood disorder neuroimaging research is the characterization of abnormalities in large-scale, distributed neural networks involved in the interface of cognitive and emotional processing. In this study, we examined brain function in 63 adolescent boys and girls with pediatric bipolar disorder (PBD) matched on mean IQ and demographics to 82 healthy controls elicited by an Affective Synonym functional magnetic resonance imaging (fMRI) task designed to probe neural networks engaged in identifying synonyms of target words. This task required attention to semantic meaning instead of distracting emotional content. Group differences in regional functional connectivity, network timecourse spectral power, and functional network connectivity (FNC) among circuits were examined using a multivariate strategy (MANCOVAN) on brain networks identified using group independent component analysis (ICA). Analyses tested whether network properties differed between diagnostic groups, gender, and their interaction, while statistically controlling for any developmental changes. Significant multivariate effects were further characterized post hoc to identify which specific brain regions, spectral frequencies, or inter-network connections had the strongest effects across specific networks. Significant diagnostic group differences in regional connectivity were found in networks found previously to be engaged for attention, language and emotion regulation - mostly PBD under-connectivity, e.g., in right middle frontal gyrus, but

some hyper-connectivity as well. Some PBD abnormalities interacted with gender, e.g., more abnormal left superior medial frontal cortex in PBD females. Greater spectral power in PBD at very low frequencies (<.05 Hz) was found only in the network ascribed to emotion regulation and also in females. Causal interactions among networks (FNC) as measured by lagged correlation found a complex array of diagnostic group differences in the degree to which the engagement of one distributed network preceded activation in others. The most pronounced gender effect is shown within the language network which exhibits increased correlations with the Valence Evaluation network. Overall, these findings show robust support for emotion-cognition network disconnection in PBD using several different but complementary analyses that assess different aspects of brain connectivity. Many of these findings depended strongly on the gender of the participant, raising important questions about specific biological contributions to abnormalities in brain network function.

Disclosures: L. Iordanescu: None. **M.C. Stevens:** None. **J. Fitzgerald:** None. **M.N. Pavuluri:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.11/EE3

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: KAKENHI C-22591265

Title: D-cell hypothesis for etiology of schizophrenia

Authors: *K. IKEMOTO;
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Abstract: Recent pharmacological discovery on trace amine-associated receptor, type 1(TAAR1) has emphasized importance of trace amines in pathogenesis of psychoses, such as schizophrenia. TAAR1 has many ligands, including tyramine, *β*-phenylethylamine (PEA), tryptamine, amphetamines, and 3'-iodothyronamine. So-called D-neuron is putative producer of trace amines, endogenous ligands of TAAR1. The D-neuron is defined "the aromatic L-amino acid decarboxylase (AADC)-containing neuron, but neither dopaminergic nor serotonergic", *i.e.* containing neither tyrosine hydroxylase nor tryptophan hydroxylase. AADC is an enzyme, also called dopa decarboxylase (DDC). The localization of D-neurons in the central nervous system has been specified into 15 groups, from the spinal cord (D1) to striatum (D15).
The author showed the decrease of D-neurons in D15 in postmortem brains of schizophrenia,

where midbrain dopamine (DA) neurons are heavily innervated. As the localization of neural stem cells of the human subventricular zone was coincide with that of striatal D-neurons (D15), the decrease of striatal D-neurons was supposed to be closely linked with neural stem cell dysfunction in schizophrenia. The reduction of striatal D-neurons may cause trace amine reduction in the striatum, and decrease stimulation of TAAR1 on striatal terminals of ventral tegmental area (VTA) DA neurons. This would increase firing frequency of VTA DA neurons, and causes mesolimbic DA hyperactivity. The author introduces a novel theory, "D-cell hypothesis", for mesolimbic DA hyperactivity of schizophrenia.
Some clinical and/or experimental evidences that support this hypothesis would be shown.

Disclosures: K. Ikemoto: None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

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Program#/Poster#: 253.12/EE4

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIMH Conte Center P50MH086383

Title: Perinatal choline as an alpha7-nicotinic receptor agonist during human fetal development

Authors: *R. FREEDMAN, R. G. ROSS;
Dept. of Psychiatry, Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: Cerebral inhibition, measured as the decrement in P50 auditory evoked response amplitude to the second of paired stimuli, is already partially developed to adult levels at the birth of most human infants. Deficits in this inhibitory sensory gating mechanism in adults are associated with schizophrenia and linked to CHRNA7, the gene for the alpha7-nicotinic receptor subunit. Parental mental illness and maternal cigarette smoking are among the factors that delay prenatal development of this function. Delayed development is associated with future problems in attention in childhood. Alpha 7-nicotinic receptor expression is one factor identified as necessary for timely development of GABA-mediated inhibition. Alpha 7-nicotinic receptors are expressed at nearly an order of magnitude higher levels in human fetal brain than in newborns or adults, but cholinergic synapses do not reach the hippocampal site of P50 generation and inhibition until shortly before birth. The endogenous ligand may be choline, present in the requisite mM concentrations in amniotic fluid. Based on positive results in a mouse model, we conducted a double-blind, randomized, placebo-controlled trial of choline supplementation at twice normal dietary levels in 100 women. Women received supplementation during the second

and third trimester, and their offspring received supplementary drops for the first 6 weeks post birth. There were no significant adverse effects. Newborns who received choline were more likely to have developed cerebral inhibition, measured as suppression of the amplitude of the second response by at least 50%, by one month, compared to infant who received placebo (76% versus 43%, Chi-square = 6.90, P = 0.009). Infants were genotyped with a SNP in the 5' promoter of CHRNA7. The minor allele previously found to be associated with schizophrenia was associated with diminished sensory inhibition in the placebo-treated newborns, but this genetic effect was not seen in choline-treated newborns. The results suggest that perinatal choline, acting as an alpha 7-nicotinic receptor agonist, may help prevent a developmental abnormality in inhibitory sensory gating associated with a portion of the genetic transmission of schizophrenia.

Disclosures: R. Freedman: None. **R.G. Ross:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.13/EE5

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: SCN2A (rs10174400) differentially modulates prefrontal activation during a fMRI processing speed task in healthy adults

Authors: *G. BAUM[1], D. DICKINSON[1], K. F. BERMAN[1], D. R. WEINBERGER[2,3], J. H. CALLICOTT[1];
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Abstract: Processing speed is a well-documented impairment in schizophrenia; argued by some to be the single strongest indicator of abnormal cognition in patients and their unaffected siblings (Knowles et al., 2012). Neuroimaging studies with healthy individuals have suggested a prominent role for the dorsolateral prefrontal cortex (DLFPFC) in mediating processing speed via functional interactions with hippocampus and motor, visual, and parietal cortices (Rypma et al., 2006). We used an event-related digit symbol substitution task (DSST), the Digit Symbol Verification Task (DSVT), using blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) with 101 healthy individuals.
Recent findings have suggested that SCN2A, a brain-specific gene that encodes the α2 subunit of

neuronal sodium channels, is strongly associated with variation in general cognitive ability (g) in schizophrenia patients (Dickinson et al., In Review). Physiologically, g has been related to the integrity and recruitment of the prefrontal cortex (PFC) (Duncan & Owen, 2000; Duncan et al., 2000). In a sample of 338 patients, SCN2A (rs10174400) was associated with DSST performance (p =.013; Dickinson, Personal Communication). Recent findings suggest that prefrontal activation during an fMRI version of the DSST is a potentially heritable phenotype in schizophrenia, such that patients and unaffected siblings of patients show reduced activation in the DLPFC relative to healthy individuals (Lau et al., In Process). In this study sample, healthy individuals' mean reaction time, the crucial variable in DSVT, varied by SCN2A genotype at a trend level (p = .077). Subjects did not differ by genotype in handedness, estimated IQ, or age. In this study we investigated the role of SCN2A in modulating prefrontal activation in healthy individuals during a processing speed task. Our findings suggest that SCN2A modulates reaction time and DLPFC activation in healthy individuals during a processing speed task, with minor allele (T) homozygotes showing significant increases in bilateral DLPFC activation relative to major (C) homozygotes and heterozygotes (R-DLPFC: pFWE = .01 SVC; L-DLPFC: -54 9 39, pFDR = .048 SVC).

Disclosures: G. Baum: None. **D. Dickinson:** None. **K.F. Berman:** None. **D.R. Weinberger:** None. **J.H. Callicott:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: Korea Healthcare Technology R & D Project Grant A101915

Title: Delayed Interplay between frontal activity and neurocardiac regulation in subjects with bipolar II disorder

Authors: *J. CHANG, K. HA, T. HA, J. HER;
Dept. of Psychiatry, Seoul Natl. Univ. Bundang Hosp., Seongnam, Korea, Republic of

Abstract: Subjects with bipolar disorders exhibit altered pattern of central autonomic function. In the frame of central autonomic network, frontal-amygdalar complex is involved in both emotion regulation and cerebral control of cardiac autonomic function. This study aimed to investigate the changes in the synchronization between frontal activity and neurocardiac dynamics in patients with bipolar II disorder during acute mental stress. Time-series data from

high-density electroencephalograms and electrocardiograms were simultaneously obtained from 40 euthymic patients with bipolar II disorder and 40 healthy controls. All the participants performed mental arithmetic task after 7 min of resting control followed by 7 min of recovery. The relationship between cardiac vagal activity (high frequency component of heart rate variability) and resting frontal alpha activity was investigated using a coherence analysis, which provides a coherence function, a gain function, and a phase shift between two time series data. Coherencies between high frequency component of heart rate variability and frontal alpha power did not differ between the bipolar II disorder and the control groups between the rest and the recovery phase. No significant difference was observed in the gain between the two groups. In contrast, values of phase shift revealed a significant phase advance of left frontal alpha activity in the bipolar II disorder group compared to the control group during the rest phase. In contrast, a significant phase delay of left frontal activity was observed in the bipolar II disorder group while performing mental arithmetic task. During recovery phase from mental stress, a greater phase delay was observed in the bipolar II disorder group compared to the control group. The results of our study suggest that the neuronal networks linking frontal activity to cardiac autonomic regulation are intact but their interaction is less efficient than that of healthy controls, implicating that patients with bipolar II disorder may exhibit maladaptive pattern of the central autonomic regulation in response to stress.

Disclosures: J. Chang: None. **K. Ha:** None. **T. Ha:** None. **J. Her:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

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UPENN brain bank.

Title: Synaptic connectivity in olfactory bulb of schizophrenia

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Abstract: Multiple lines of evidence suggest altered synaptic plasticity/connectivity as a pathophysiologic mechanism for various symptom domains of schizophrenia. This notion is supported by the findings that patients with schizophrenia exhibit abnormalities in neurocognitive function and changes in EEG and brain imaging parameters that may be associated with synaptic connectivity. Moreover, postmortem studies offer further support by demonstrating ultra-structural changes in synapses, i.e., decreased dendritic spine densities, as well as altered expression levels of synaptic proteins. Among endophenotypes of schizophrenia is olfactory dysfunction, which is manifested by decreases in olfactory sensitivity, discrimination and memory. Electrophysiological underpinnings of these abnormalities have also been demonstrated by decreased odorant induced evoked potentials (EP) in the patient group. The olfactory circuit is based on a mono-synaptic connection in the glomeruli of the olfactory bulb, which conveys signals from olfactory receptor neurons to the olfactory cortex. We hypothesized that olfactory dysfunction and decreased olfactory EP in schizophrenia is associated with dysregulations in synaptic in the glomeruli of the olfactory bulb. To test this, we have examined the olfactory bulbs of 11 patients with schizophrenia and their age- and sex-matched pairs for the expression of 5 pre- and post-synaptic molecules in the glomeruli. Olfactory bulbs, paraformaldehyde fixed, were examined by quantitative histologic assessment of the immunoreactivity detected by DAB. The intensity of signals was corrected for the background and measured as optical density using Image-Pro 7.0 software. We found significant decreases in PSD-95, a scaffolding protein in the PSD, in the glomeruli of schizophrenia cases compared to their matched controls (-34.06%, p=0.015, two tailed t test paired). Spinophilin and Synaptopodin play critical roles in modulating the morphology and activity of dendritic spines. The SCZ group showed significant decreases in Spinophilin (-18.07%, p=0.043, two tailed t test paired). In addition, we found decreases in Synaptophysin (-23.94%, p=0.003, two tailed t test paired), which targets the vesicles to the plasma membrane was also decreased in the SCZ group. Together, our results present preliminary evidence for altered synaptic connectivity in schizophrenia and demonstrate that the glomeruli of the olfactory bulb is a structure that may reveal disease related alterations in synaptic connectivity.

Disclosures: C.N. Egbujo: None. **K. Borgmann-Winter:** None. **S. Arnold:** None. **K. Talbot:** None. **B. Turetsky:** None. **C. Hahn:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

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DOE Grant DE-FG02-99ER62764

Title: Potential influence of miR-137-associated rs1625579 genotype on structural brain variation in schizophrenia

Authors: V. PATEL[1], N. COTA[1], C. WRIGHT[2], N. I. PERRONE-BIZZOZERO[2], M. MORGAN[1], V. D. CALHOUN[1], T. WASSINK[3], B. HO[3], S. EHRLICH[4], J. HASS[4], K. ALPERT[5], L. WANG[5], *J. A. TURNER[1];
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Abstract: Objective. The single nucleotide polymorphism (SNP) rs1625579 in the miR-137 host gene has recently been shown to be associated with risk of developing schizophrenia (SZ). In vivo animal studies suggest that miR-137 and select targeted genes may also regulate neuronal development. A previous study into the role of rs1625579 on structural brain volumes observed larger lateral ventricles and smaller hippocampal volumes in SZ patients who were homozygous for the risk allele. We examined potential effects of the miR-137-associated SNP genotype on subcortical brain volume in SZ patients versus controls.
Methods. Our sample was combined across two legacy datasets, comprising in total 370 individuals (185 SZ patients, 244 males), genotyped for rs1625579 and with subcortical volumes from T1-weighted MRI scans (segmented using Freesurfer 4.5 and 5.x). We performed a MANCOVA analysis to examine the relationship between SNP genotype and diagnosis on 35 volumes. We used genotype as a factor with two levels, and assigned data collection site, age, gender, and intracranial volume as covariates. Due to the low incidence of homozygous non-risk allele carriers, these and heterozygous individuals were combined in the genotype factor.
Results. Our analysis yielded a significant main effect of SNP genotype only on the volume of the right inferior lateral ventricle. The number of risk alleles corresponded to higher ventricle volume in both patients and controls. We also observed significant genotype by diagnosis interactions in the bilateral putamen, right thalamus, and right caudate. In all three regions, there was a positive relationship between number of risk alleles and volume in SZ patients, and a negative relationship in controls.
In a 228 subject subset of the data (110 SZ patients, 154 males) that included corpus callosum

and striatal volume measures, an additional MANCOVA revealed significant genotype by diagnosis interactions on the volume of the anterior corpus callosum and right dorsal striatum, with a near-significant effect on the left dorsal striatum. In the anterior corpus callosum, the number of risk alleles was positively related to volume in patients and negatively related in controls. This relationship was maintained in both the left and right dorsal striatum. Conclusions. Our results suggest a potential role of the rs1625579 genotype in subcortical volumes of SZ. While many of these subcortical areas show volume loss in SZ, the effects of the SNP genotype could impact certain regions differently across diagnostic groups. This may bear relation to the role of miR-137 in the regulation of neuronal development in the brain.

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Poster

253. **Schizophrenia: Altered Brain Network Function**

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: Support from Otsuka Pharmaceutica

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Title: Difference of reward system activation in patients with schizophrenia treated with olanzapine, blonanserine and aripiprazole-2nd report

Authors: *N. HASHIMOTO, A. TOYOMAKI, T. MIYAMOTO, I. KUSUMI;
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Abstract: In schizophrenia, unmedicated patients or patients medicated with typical antipsychotics, confronted with reward-indicating stimuli displayed a reduced activation of the ventral striatum. On the other hand, patients treated with atypical antipsychotics showed ventral striatal activation in response to reward-indicating cues. However, to our knowledge, an effect on ventral striatal activation in reward-indicating stimuli of the atypical antipsychotics other than olanzapine (OLZ), risperidone and amisulpiride have never been studied. Therefore, we examined schizophrenia patients treated with OLZ, blonanserin (BLN) and aripiprazole (ARP), and healthy controls by measuring ventral striatal activation during a monetary incentive delay (MID) task. Methods: The local ethics committee of Hokkaido University approved the study,

and written informed consent was obtained from all participants. Twenty six schizophrenics and fourteen healthy volunteers participated in a fMRI study performing a MID task. In this task incentive cues predicting monetary gain (200, 50), no gain (0) or uncertain gain (???), target stimuli, and feedback stimuli were presented. Participants were requested to response as quick as possible against target stimuli and would be notified the result of their responses by feedback stimuli. Results: In a behavioral level, reaction time was significantly longer in schizophrenia group than that in control group, but no difference was observed in the hit rate. The region of interest analysis using a two-way ANOVA [two contrast factors("200 vs 0", "50 vs 0") x two diagnosis factors or two contrast factors x three main antipsychotic factors (OLZ, BNS, ARP)] revealed a significant main effect of contrast for the activation in the bilateral ventral striatum["200 vs 0" showed significantly higher activation than "50 vs 0" in the both ventral striatum]. There was no main effect of diagnosis and interaction of contrast x diagnosis. However, we found a significant interaction of contrast x antipsychotics in the bilateral ventral striatum. Conclusion: OLZ, BNS and ARP would improve disturbance in ventral striatum reward system in schizophrenia patients. OLZ and BNS might be better to improve the ventral striatum reward system disturbance than ARP.

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Poster

253. **Schizophrenia: Altered Brain Network Function**

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Cortical EEG information flow in schizophrenics and controls during resting state

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Abstract: Background
Research on schizophrenia has failed to find physical biomarkers of the disease. An alternative

approach is to consider dynamics of brain activity within large-scale cortical circuits. Here we report first results of a source-resolved, high-density EEG study of cortical network information flow in schizophrenia patients and control subjects during rest.

Materials and Methods

47 subjects (27 schizophrenia, 20 control) participated in the study. EEG data were recorded from 68 scalp sites while the seated subjects sat relaxed with eyes open for 5 minutes. Data were processed using software tools from EEGLAB (sccn.ucsd.edu/eeglab). After cleaning the data using methods from the BCILAB toobox (sccn.ucsd.edu/wiki/BCILAB), the data were decomposed into maximally independent component sources using adaptive mixture ICA (AMICA). Brain-based sources were localized by equivalent dipole, and information flow between them was measured using SIFT toolbox (sccn.ucsd.edu/wiki/SIFT) methods based on multivariate vector autoregression. Total renormalized partial directed coherence (rPDC) values, summing spectral information inflow to and outflow from each localized source process, were submitted to Measure Projection (sccn.ucsd.edu/wiki/MPT) for group-level imaging.

Results

Measure projection found a spatial domain in or near mid-cingulate cortex within which schizophrenia patient sources showed significantly more low-frequency information outflow (1-7 Hz), and a spatial domain in or near anterior cingulate cortex in which patients showed significantly less high-frequency information inflow (above 90 Hz).

Conclusion

Cortical network analysis during rest revealed that schizophrenia patients and control subjects at rest have distinguishable patterns of functional EEG connectivity in mid-cingulate and anterior cingulate cortex. These findings are in harmony with previous research and demonstrate the utility of EEG information flow analysis in psychiatric studies.

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Poster

253. **Schizophrenia: Altered Brain Network Function**

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Title: Small saccades and image complexity during free viewing of natural images in schizophrenia

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Abstract: Abnormalities in persons affected with schizophrenia encompass a variety of brain cognitive processes, including eye movement's control, which require extensive resources from the brain. Patients display dysfunctions during the execution of simple visual tasks such as anti-saccade or smooth pursuit. In more ecological scenarios, such as free viewing of natural images, patients appear to make fewer and longer visual fixations and display shorter scanpaths. It is not clear whether these measurements reflect alterations in their proficiency to perform basic eye movements such as saccades and fixations or are related to higher-order brain mechanisms, such as visual exploration or attention. We utilized free
exploration of natural images of different complexity as a model of an
ecological context where normally operative mechanisms of visual control can be accurately measured. We quantified visual exploration, scanpaths, saccades and visual fixation, using the standard SR-Research eye tracker algorithm (SR) and compared this result with a computation that include saccades and microsaccades (EM). We evaluate 8 schizophrenia patients and corresponding healthy controls and tested whether the decrement in the number of saccades and fixations, as well as their increment in duration reported previously in schizophrenia patients, resulted from the increasing occurrence of undetected small saccades. We found that when utilizing the standard SR algorithm, patients display shorter scanpaths as well as fewer and shorter saccades and fixations, showing significant difference with the control group. When we employed the EM algorithm, difference in these parameters between patients and healthy controls were no longer significant. On the other hand, we found that content of images plays an important role in exploratory behaviors.
These results contribute to elucidate the mechanisms of visual motor control that are affected in schizophrenia and contribute to the finding of adequate markers for diagnosis and treatment for this condition.

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Poster

253. **Schizophrenia: Altered Brain Network Function**

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Program#/Poster#: 253.20/EE12

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Significant changes in local connectivity in early-onset bipolar disorder with psychosis

Authors: *H. M. FERNANDES[1,2], J. CABRAL[1,3], M. PETERSEN[1,2], T. J. VAN HARTEVELT[1,2], A. C. JAMES[4], G. DECO[3,5], M. L. KRINGELBACH[1,2];
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Abstract: Pediatric bipolar disorder (PBD) with psychosis (delusions and/or hallucinations) is currently not well understood in terms of its underlying neurobiology. The diagnosis of PBD is based primarily on taking clinical history, which is considerably more difficult to obtain from children and adolescents than from adults. The clinical diagnosis could potentially benefit from a better understanding of the underlying neurobiology of PBD, and in particular of the early structural changes in connectivity.
In the current study, we examined the changes in cognitive scores and structural connectivity in a group of 15 adolescents with PBD and psychosis compared to a 25 euthymic matched healthy controls, having constructed the connectomes using probabilistic tractography and diffusion tensor imaging.
We found significant differences in the structural connectivity of anterior regions within the so-called ‘default mode’ network including regions of the medial orbitofrontal cortices and precuneus. These results show that PBD is associated with changes in structural connectivity in regions involved in brain networks associated with emotional processing and regulation.

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Poster

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Title: Disrupted slow wave modulation of spindle oscillations during Non-REM sleep correlates with procedural memory deficits in patients with schizophrenia

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Abstract: Abnormalities in sleep EEG have been linked to the severity of cognitive deficits in schizophrenia. A recent study identified a decoupling of characteristic EEG/LFP oscillations during NREM sleep in an animal model of schizophrenia (Phillips, Bartsch et al. Neuron 76 (3): 526-533, 2012).
Motivated by the results from the animal model we re-analysed a patient dataset in which reduction of spindle amplitude and coherence was linked to procedural memory deficits (see Wamsley et al., Biol Psychiatry 71 (2): 154-161, 2012). We particularly focused on the interaction of slow (0.5-1.5Hz) and spindle oscillations (10-15 Hz) during NREM sleep, since these have been implicated in memory consolidation in both humans and rodents.
Our new analysis revealed a slight reduction in overall slow wave amplitude evident in wave triggered averages of detected slow waves. To assess coordination of spindle oscillations relative to slow waves, we computed moving window multi-taper spectrograms and compared local slow wave triggered spindle power at all recording sites. We found that spindle power is strongly modulated by slow waves in control subjects, but this modulation is markedly reduced in schizophrenia patients.
We next computed slow wave triggered coherograms to quantify remote coupling of frontal to parietal and central to occipital regions during slow wave events. We found strong spindle coherence after slow waves in the control group, and this coherence increased significantly after learning but was significantly attenuated in patients. In contrast, coherence between spindles not associated with slow waves (at least 3 seconds outside any slow wave activity) was less severely reduced and showed no learning-dependent increase in patients. A regression analysis confirms that slow wave modulated spindle coherence is significantly correlated with overnight motor task improvement; whereas non-slow wave modulated spindle coherence shows no such correlation.

In summary we confirm findings from an animal model of schizophrenia in a patient dataset, showing reduced frontal to occipital coupling during NREM sleep. We propose slow wave-spindle interactions as a new EEG based translational biomarker that implicates thalamocortical circuit dysfunction in cognitive symptoms of the disease.

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Poster

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIH Grant R01MH083968

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Title: Temporal stability of default mode network in euthymic bipolar patients

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Abstract: Disturbances in functional connectivity have been suggested to contribute to cognitive and emotion processing deficits observed in bipolar disorder. Functional connectivity between medial prefrontal cortex (MPFC) and other brain regions may be particularly abnormal. The goal of the present study was to characterize temporal dynamics of the default mode network during rest in bipolar disorder using high resolution sequences adapted from the Human Connectome Project.
Bipolar participants (N=13, age 50.7±12.1) were medicated and euthymic at the time of participation (HAM-D = 4.0±2.7, YMRS = 1.0±1.7, PANSS Total =37.0±5.9). Healthy comparison participants (N=14, age 53.3±10.8) were comparable in age, gender, and education. Participants were given a cognitive battery focused on executive function including DKEFS Trail Making and Color Word Interference and rated their sleepiness and anxiety prior to the fMRI scan. Ten minutes of 2x2x2 mm multi-band gradient echo data were collected at a repetition time of 720 ms while participants rested quietly in a 3T GE MR750 scanner. We used a seed-based approach to measure default mode network correlations in each participant with seeds placed in

the MPFC, posterior cingulate, and lateral parietal cortex (Fox et al, 2005). We then divided the continuous scan into eight epochs and calculated average connectivity between MPFC and other default mode nodes for each epoch.
The bipolar group performed worse on Trails Number Sequencing and Letter Number Switching and Color Word Interference/Switching tasks suggesting slight impairment in visual attention and executive function. In addition, bipolar patients' self-rated anxiety level was higher at the time of the scan. Functional connectivity between MPFC and other cortical regions was slightly decreased in the bipolar group. Importantly, within-subject temporal variability in connectivity between MPFC and other default network regions was reduced in bipolar group.
The preliminary results suggest that default mode network connectivity in euthymic bipolar patients appears to be more stable across a ten minute period. Further explorations will be needed to assess the relationship between temporal stability of default mode network connectivity and other factors such as psychotropic medication.

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Poster

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Distinguishing 1H-MRS metabolites in schizophrenia and methamphetamine-induced psychotic disorder

Authors: J. HSIEH, H. TEMMINGH, D. J. STEIN, *F. HOWELLS;
Dept. of Psychiatry, Univ. of Cape Town, Cape Town, South Africa

Abstract: The neurobiological mechanisms that result in the development and persistence of psychotic disorders are currently unknown. Methamphetamine-induced psychosis (MAP) may provide useful insights into the neurobiology of psychotic processes. Magnetic resonance spectroscopy (MRS) provides an in vivo assessment of brain metabolites, and has not previously been used to compare similarities and differences in schizophrenia and MAP in regions putatively involved in these disorders.1H-MRS was used to measure absolute metabolites in individuals with schizophrenia, MAP, and healthy socio-demographic controls. Metabolites were measured in left dorso-lateral prefrontal cortex, anterior cingulate cortex, and left thalamus. Our results revealed both similarities and differences in MRS metabolites in schizophrenia and MAP.

In particular NAA was decreased in anterior cingulate in both schizophrenia and MAP compared to controls, while schizophrenia was characterized by increased glutamate and glutamine (glx) in thalamus compared to healthy controls. Both schizophrenia and MAP involve alterations in neuroplasticity with evidence of decreased neuronal integrity and viability, as indicated by decreased NAA in anterior cingulate cortex. However, schizophrenia may be characterized by more severe disruptions in cortico-thalamic structures, as indicated by increased glutamate and glutamine in the thalamus.

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Poster

253. **Schizophrenia: Altered Brain Network Function**

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Title: Localized changes in white matter connectivity in early-onset schizophrenia and its functional consequences

Authors: *J. CABRAL[1,2], H. M. FERNANDES[2,3], T. J. VAN HARTEVELT[2,3], A. C. JAMES[4]; [1]Ctr. of Brain and Cognition, Barcelona, Spain; [2]Dept of Psychiatry, Univ. of Oxford, Oxford, United Kingdom; [3]Ctr. of Functionally Integrative Neurosci., Aarhus Univ., Aahrus, Denmark; [4]Warneford Hosp., Oxford, United Kingdom

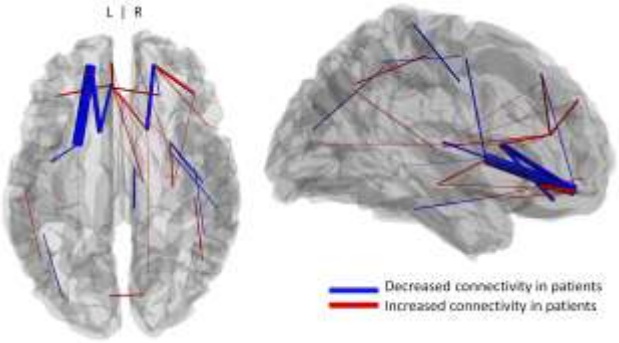
Abstract: Early-onset schizophrenia is a severely disabling illness which is currently not well understood in terms of its underlying neurobiology. However, emerging evidence suggests that schizophrenia is strongly linked to problems with cortical connectivity.

In the current study, we compared the structural connectivity between 90 brain areas of a group of 20 adolescent patients with early-onset schizophrenia with a group of 20 age-matched healthy controls. The structural connectomes were constructed from diffusion tensor imaging data using probabilistic tractography and analyzed using statistical tests and measures from graph theory. Subsequently, we used a computational model to gain insight on the functional impact of the observed structural alterations on the large-scale brain dynamics of patients and healthy controls. In the model, each brain area was represented by a dynamic mean field model, receiving excitatory input from connected areas in proportion to the number of white matter fibers detected between them.

We found significant changes in structural connectivity between patients and controls. Significant decreases were observed in primarily left lateralized anterior networks centered around the left orbitofrontal and anterior insular cortices. In addition, the connectivity between the superior orbito-frontal cortex and the caudate gyrus, exhibited a significant decrease in both the left and right hemispheres in schizophrenia. We also found significant increases in connectivity between the left cingulate cortex and right thalamus, right caudate and right superior frontal cortex.

Our computational model indicates that the brain in schizophrenia operates with lower global coupling strength, which shifts the dynamics from the optimal healthy regime to a point where functional networks appear subtly randomized and less small-world when compared to healthy participants.

Results show that schizophrenia is associated with lateralized changes in structural connectivity in regions involved in brain networks associated with emotional processing and regulation.



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Poster

253. **Schizophrenia: Altered Brain Network Function**

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Title: Anterior but not posterior hippocampal volume is reduced in schizophrenia

Authors: *P. A. TALATI[1], A. LUKSIK[1], C. KONRADI[2], S. HECKERS[1];
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Abstract: MRI studies have consistently reported smaller bilateral hippocampal volume in schizophrenia. However, it is unclear whether the volume loss is diffuse or localized to one region of the hippocampus. Manual segmentation of the hippocampus in 83 patients with schizophrenia and 78 controls matched for age, gender and race, revealed a significant region by diagnosis interaction, with significantly smaller anterior, but normal posterior, hippocampal volume bilaterally in schizophrenia. From a candidate list of six genes known to affect hippocampal volume, we investigated whether single nucleotide polymorphisms (SNPs) can account for the lower anterior hippocampal volume in schizophrenia. Our results indicate that the BDNF Val/Val (rs6265), MIR137HG AC/CC (rs1625579), and the TNF GG (rs1800629) alleles each account for the hippocampal volume differences in our sample. We then investigated functional consequences of smaller anterior hippocampal volume. Specifically, we studied whether the normal anterior right > left hippocampal volume asymmetry was maintained in schizophrenia. We found the normal anterior hippocampal asymmetry in schizophrenia, which correlated with verbal fluency and processing speed when controlling for age, gender, and group. Future studies should explore further how smaller anterior but normal posterior hippocampal volume can explain the clinical features of schizophrenia.

Disclosures: P.A. Talati: None. **A. Luksik:** None. **C. Konradi:** None. **S. Heckers:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.26/EE18

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIMH K08

MH080329

P50 MH084053

Title: Synchronization and schizophrenia: What about cross frequency coupling disturbances?

Authors: *N. POLIZZOTTO[1], C. WALKER[2], T. WOZNY[2], R. Y. CHO[2];
[2]Psychiatry, [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Study: Brain rhythm disturbances are often observed in schizophrenia and are typically reported as independent or merely co-occurring alterations of both high and low frequency activity. However functional integration of activities occurring across different frequencies is an important feature in the healthy brain. In particular phase-amplitude coupling (PAC), i.e. the modulation of high frequency amplitude by the phase of low frequencies, has been suggested to be functionally relevant to perception and cognition and possibly providing an integrative account of wide spectral alterations in schizophrenia (Lisman, 2008). Only 2 studies have empirically addressed this hypothesis to date (Spencer, 2009; Kirihara, 2012). Critical methodological differences (e.g. different frequencies of interest), and analytical problems intrinsic to PAC estimations (e.g. dependence on amplitude) do not allow reconciling of their disparate results. We aimed to provide a more rigorous and comprehensive account of PAC in schizophrenia patients, investigating PAC across a broad range of EEG bands, together with its relation to amplitude differences.
Methods: Cross-frequency coupling analysis was applied to previously published eeg data (Kömek, et al 2012), where 12 schizophrenia vs. 12 healthy controls (age 30.3 ±9.5 vs. 31.4 ±9.1, gender and parental education matched) performed an auditory entrainment paradigm. Modulating phase and modulated amplitude were analytically extracted as angle and magnitude of the wavelet transformed time series. Phase-amplitude interaction was assessed by mutual information between their distributions.
Results: Schizophrenia subjects had impaired alpha-gamma coupling compared to healthy controls. This decreased coupling was found in the context of increased alpha power and decreased gamma power in patients compared to controls, indicating that coupling strength cannot be predicted simply by activity patterns at the respective frequencies. Other PAC alterations were observed possibly related to amplitude differences between populations, for instance, increased gamma power higher in controls and theta power in patients.
Conclusion: Observations were made in the context of a sensory entrainment paradigm and spectral power differences between populations. However this finding provides preliminary grounds for claims that pathophysiologic mechanisms in schizophrenia can involve coupling disturbances, over and above amplitude alterations within individual bands and highlights the importance of assessing coupling alterations across a broad frequency spectrum.

Disclosures: N. Polizzotto: None. **C. Walker:** None. **T. Wozny:** None. **R.Y. Cho:** None.

Poster

253. Schizophrenia: Altered Brain Network Function

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.27/FF1

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Functional connectivity in euthymic bipolar disorder reveals increased dynamic range

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Abstract: While euthymic bipolar subjects (BPE) do not experience the emotional turmoil of mania and depression, impairment of sustained attention [3], verbal memory and executive function [2] persist. Additionally recent work has shown that BPE subjects have hyper-connected cortico-limbic connections [4] during resting state. This work explores functional connectivity (FC) further by comparing whole brain FC during resting state and an emotion matching task. MPRAGE,fMRI data was carried out on a Siemens Trio 3T scanner (TR 2s) and segmented using Freesurfer. 21 control (10M, 11F) and 24 euthymic subjects (12M, 12F) were scanned during resting state and during an emotion matching/recognition task [1]. Data were motion corrected and independent components from non-grey matter voxels regressed out. FC differences between bipolar and normal subjects were controlled for motion, age, sex and brain size. False discovery rate was controlled for (fdr < 0.1). During the emotion identification task, BPEs showed greater overall connectivity (across all regions, t = 316), whereas the opposite was true for resting state (t = -154).
Functional connections most impaired during resting state includes right Jensen's sulcus (S.) with 1) right circular S. of the insula, 2) the right lingual gyrus (G.), 3) the right anterior cingulate, 4) the right postcentral G., 5) both calcarine sulci, 6) the left middle frontal S., 7) the left hippocampus, 8) the left superior frontal G., 9) the left thalamus and 10) the left inferior frontal G. (IFG). The Left Posterior-ventral cingulate was also significantly less connected to 1) the right postcentral S. , 2) the right subcallosal area, and 3) the left superior temporal G. During the task bipolar subjects showed significantly increased connectivity between the left suborbital S. and 1) the right orbital part of the IFG 2) the right lateral orbital S. 3) right medial orbital S. 4) the left orbital G./S. 5) left postcentral S. The right lateral orbital sulcus and 1) the left orbital S. and 2) the left anterior segment of the lateral S. were also significantly more functionally connected.

These results confirm that bipolar subjects continue to show dis-regulation of brain connectivity during euthymia and shows that this manifests itself as both hyper-connectivity and hypo-connectivity depending on the functional task.
[1] L. Altshuler et al. The American journal of psychiatry, 162(6):1211-3, June 2005.
[2] L. J. Robinson et al. Journal of affective disorders, 93(1-3):105-15, July 2006.
[3] G. Sepede et al. Bipolar disorders, 14(7):764-79, Nov. 2012.
[4] S. Torrisi et al. Bipolar disorders, 15(2):156-66, Mar. 2013.

Disclosures: M.C. Chambers: None. **J.D. Van Horn:** None. **L. Altshuler:** None. **G. Bartzokis:** None. **C. Torgerson:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.28/FF2

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NSFC Grant 30870766

NSFC Grant CB707805

Title: Slow binocular rivalry rate in patients with bipolar disorder,OCD,major depression,and schizophrenia

Authors: *X. YE[1], R. ZHU[1], K. WANG[1], S. HE[2];
[1]Dept. of Neurol., The First Affiliated Hosp. of An Hui Med. Univ., An Hui, China; [2]Dept. of Psychology, Univ. of Minnesota, Minneapolis, MN

Abstract: When two different images are presented to the two eyes dichoptically, observers usually experience a perceptual alternation between the two images. This phenomenon, known as binocular rivalry, has been used as a powerful tool to investigate mechanisms of visual awareness. It was also found that the rates of perceptual alternation are slower in patients with bipolar disorder than that in normal controls (Pettigrew & Miller, 1998). To investigate the broader clinical relevance of binocular rivalry in mental disorders, we measured the perceptual alternation rates during rivalry in normal controls (n=30) as well as patients with different types of psychiatric disorders, including bipolar disorder type I (n=30), obsessive compulsive disorder (OCD, n=21), major depression (n=46), schizophrenia (n=48), and first-degree relatives of schizophrenics (n=31). Participants viewed competing red-green images on computer monitor through red-green anaglyph glasses and pressed one of two buttons to record their alternating

perception. The distribution of normalized rivalry intervals was well described by a gamma function in all groups, suggesting that participants indeed experienced binocular rivalry. Critically, the median rate of perceptual alternation was 0.28 Hz for bipolar patients, 0.17 Hz for OCD patients, 0.23 Hz for major depression patients, 0.20 Hz and 0.24 Hz for schizophrenia patients and their first-degree relatives respectively, all significantly slower than the rate of 0.46 Hz obtained from the normal controls.
Our results show that binocular rivalry is slower in several groups of patients with mental disorders. It is possible that the abnormal temporal dynamics of binocular rivalry in patient groups may serve as a potential endophenotype for these mental disorders. However, the fact that different types of mental disorders all demonstrated similarly slower rivalry alternation suggest that the mechanism responsible for the slowdown in perceptual switching is not disease-specific. On the other hand, the current results are consistent with the possibility that these different mental disorders may have shared genetic roots. The finding that first-degree relatives of schizophrenics also had slower rivalry alternation provides further support that there might be a common genetic component in schizophrenia and in the mechanism that determines the temporal dynamics of rivalry.

Disclosures: X. Ye: None. **R. Zhu:** None. **K. Wang:** None. **S. He:** None.

Poster

253. **Schizophrenia: Altered Brain Network Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 253.29/FF3

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: LOWEgrantNeuronaleKoordinationForschungsschwerpunkt Frankfurt(NeFF)

Title: Effects of ketamine on resting-state and task-related neural oscillations in MEG-data

Authors: *D. RIVOLTA[1], A. SAUER[2], T. HEIDEGGER[3], K. BIRKNER[2], B. SCHELLER[3], M. WIBRAL[3], W. SINGER[2], P. J. UHLHAAS[4];
[1]Max Planck Inst. For Brain Res., Frankfurt Am Main, Germany; [2]Max Planck Inst. for Brain Res., Frankfurt am Main, Germany; [3]Goethe Univ., Frankfurt am Main, Germany; [4]Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Aberrant neural oscillations in the gamma-band range (> 30 Hz) are crucially involved in the pathophysiology of schizophrenia. A possible model for dysfunctional high-frequency oscillations involves the disrupted glutamatergic neurotransmission mediated by the N-methyl-D-aspartate (NMDA) receptor. In the current study, we examined the effects of NMDA-receptor

hypofunctioning on gamma-band activity during the administration of ketamine to healthy volunteers. Neural oscillations were recorded using a 275-sensors Magnetoencephalography-(MEG) system in a group of 15 healthy volunteers during the administration of a sub-anesthetic dose of ketamine (0.006 mg/Kg) and a placebo saline solution in a single-blinded within-subject design. For each participant, we recorded oscillations induced by sinusoidal gratings (5° of visual angle) and during rest. Data were analyzed at the sensor and source level (i.e., beamforming approach) by extracting time frequency series (1-120 Hz). Ketamine, compared to placebo, led to an increased visually-induced gamma band oscillations (45-75 Hz) over occipital sensors, with sources localized to early visual areas. Ketamine also increased gamma-activity (30-90 Hz) at rest over fronto-central sensors, with sources showing the strongest effect localized in the right hippocampus, thalamus (bilateral) and left anterior cingulate cortex. The acute administration of ketamine leads to an upregulated gamma-band activity both at rest and during visual processing. This is possibly mediated by a shift in the excitation/inhibition balance in favor of excitation of pyramidal cells due to hypofunctioning NMDA-receptors. Since the upregulation of gamma-band activity has been described in early psychosis, our results support the clinical relevance of the NMDA-receptor hypofunctioning model of schizophrenia.

Disclosures: D. Rivolta: None. **A. Sauer:** None. **T. Heidegger:** None. **K. Birkner:** None. **B. Scheller:** None. **M. Wibral:** None. **W. Singer:** None. **P.J. Uhlhaas:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.01/FF4

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NARSAD Grant Hippocampal Learning and Memory Mechanisms in Schizophrenia

NIMH Grant MH062236 Examination of the Lymbic Cortex in Schizophrenia

Title: Reverse translation animal model of hippocampal dysfunction in Schizophrenia

Authors: *S. SOUTHCOTT, M. YANAGI, J. LISTER, I. LEE, C. TAMMINGA;
Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Animal models for psychosis have been inadequate because the cellular and molecular characteristics of the condition itself have been obscure. As the disease neurobiology is advancing, it is becoming possible to develop animal models through reverse translation, mimicking the biology of the human condition. We have focused on modeling hippocampal

pathology and its learning and memory component as it informs psychosis. In clinical studies of psychosis in schizophrenia, we show hippocampal hyperperfusion, GluN1 protein reduction, particularly in dentate gyrus (DG) and synaptic strengthening at the NIMDA receptor in CA3 along with alterations in relational memory capacity in the illness (Tamminga, et al., 2010 and 2012; Li, et al., 2012); these are the molecular and behavioral targets we have modeled in the animal. To explore a reverse translation animal model, we crossed a POMC-Cre mouse line with a floxed P-GluN1 mouse to create a DG-specific knock down of GluN1 protein in the animal. These animals have reduced levels of GluN1 restricted to DG (WT n=7; KO n=7). Behaviorally, these mice show decreased pre-pulse inhibition, reduced learning in the Morris Water Maze, increased freezing in a fear conditioning paradigm (Contextual FC p=0.04; Cued FC p=0.004) and an increased latency to respond in the passive avoidance paradigm (p=0.01). In tissue, we met the tissue phenotype of reduced GluN1 in DG but failed to generated the psychosis fingerprint of increased synaptic strengthen marker in CA3 at the NMDA receptor. However, we modified this animal model in several ways and have, in the end, been able to adapt this KO animal to show both the behavioral phenotype of psychosis (noted above) and molecular evidence reflecting both the DG GluN1 reduction and the psychosis finger-print (increased GluN2B: WT n=7; KO n=7; p=0.035) in CA3. We will include the full animal behavioral profile and the tissue bio-signature of this psychosis animal model.

Li W, Potts B, Perez J, Ghose S, Tamminga C. Examining learning and memory plasticity in hippocampal subfields in schizophrenia. Poster session presented at: SFN 2012. Oct 13-17 2012; New Orleans, LA.

Tamminga CA, Southcott S, Sacco C, Wagner AD, Ghose S. Glutamate dysfunction in hippocampus: relevance of dentate gyrus and CA3 signaling. Schizophr Bull. 2012 Sep;38(5):927-35.

Tamminga CA, Stan AD, Wagner AD. The hippocampal formation in schizophrenia. Am J Psychiatry. 2010 Oct;167(10):1178-93.

Disclosures: S. Southcott: None. **M. Yanagi:** None. **J. Lister:** None. **I. Lee:** None. **C. Tamminga:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.02/FF5

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Evidence of hippocampal ca3-specific nmda receptor pathology in psychosis in schizophrenia

Authors: *C. A. TAMMINGA[1], W. LI[2], J. PEREZ[3], B. POTTS[4], C. MEYER[4], S. ARIAGNO[4], S. GHOSE[2];
[1]Univ. Texas Southwestern Med. Ctr., Dallas, TX; [2]Psychiatry, [3]Psych, [4]UT Southwestern Med. Ctr., Dallas, TX

Abstract: Schizophrenia has no known cellular or molecular pathophysiology, thus putting it at great disadvantage with respect to diagnostic considerations and therapeutic drug development. The most florid and unusual of its clinical dimensions is psychosis. In psychosis, the brain generates false, persistent and unpleasant perceptions (hallucinations) and false beliefs (delusions) much like psychotic memories. We have proposed a learning and memory model of psychosis, which is based on early evidence of increased perfusion in schizophrenia hippocampus, along with reduced glutamate signaling in dentate gyrus (AmJPsych 167:1178, 2010); therefore, we have been testing CA3 not only for evidence of increased *in vivo* function but also for *in vitro* tissue correlates of increased synaptic strength. The increase in CA3 function plausibly could generate vulnerability for mistakes of association and could mediate their encoding as false memories, even those with psychotic content. To test this model, we have carried out CA3-specific analyses of *in vitro* postmortem tissue using molecular markers of synaptic strength. We postulated an increase in CA3 perfusion downstream to CA1 and increase in markers of synaptic strength limited to CA3. We report an increase in GluN2B/GluN1 in CA3 ($p=0.009$) but fail to find any change in GluN2B/GluN1 in CA1 ($p=0.34$). Consistent with the increase of this protein in CA3 we also identify elevation of PSD-95, a change that is only apparent in CA3 ($p=0.01$). These changes were sustained in tissue drug-free at the time of death, confirming them as associated with disease, not medication. GAD-67 protein was unchanged in CA3 ($p=0.64$) and in CA1 ($p=0.698$). In an exploratory analysis of related synaptic proteins, we show a weak increase in GluN2A/GluN1 $p=0.05$) and a trend toward an increase in SAP-102 ($p=0.07$) all in CA3, and with the P-CREB/CREB significantly decreased in CA1 ($p=0.05$). We interpret these findings to confirm our hypothesis of an increase in synaptic strength in CA3 with increased neural activity in the Schaffer Collaterals onto CA1 neurons and suggest that this is mediated through increased number and sensitivity of the postsynaptic NMDA receptors in CA3. We propose that this increase in synaptic strength creates a risk state for psychosis which can be overwhelming in itself or can be activated by separate afferents (perhaps representing stress-related signaling) which act on high synaptic strength in CA3 to generate a ‘run away’ positive feed forward signaling within the recurrent collateral pathways in CA3. This could pervert normal associational activity in hippocampal CA3 (ProgBrainRes 169:225, 2008) and generate psychotic phenomena.

Disclosures: C.A. Tamminga: A. Employment/Salary (full or part-time):; Full Time at UT Southwestern Medical Center. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a

drug study, report that research relationship even if those funds come to an institution.; Drug study with Sunovion Pharmaceuticals. F. Consulting Fees (e.g., advisory boards); Eli Lilly, Lundbeck, Inc, Astellas, Intra-cellurlar Therapies. **W. Li:** None. **J. Perez:** None. **B. Potts:** None. **C. Meyer:** None. **S. Ariagno:** None. **S. Ghose:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

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Program#/Poster#: 254.03/FF6

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: mH 062236

mH 83957

NARSAD

Title: Whole-transcriptome analysis of hippocampal CA3 in schizophrenia

Authors: *J. M. PEREZ, K. GLEASON, S. GHOSE, T.-K. KIM, C. TAMMINGA;
UT Southwestern, Dallas, TX

Abstract: Schizophrenia (SZ) is one of the thirty most incapacitating conditions in the world and affects over 67 million people worldwide; suicide occurs in 10% of those diagnosed with schizophrenia. Symptoms are persistent and often severe. They include hallucinations, delusions, thought disorder, and deficits in executive function and memory. Treatments available are not always efficacious. 20-40% of people with schizophrenia are resistant to treatment and less than 20% completely recover after one episode of psychosis. Due to a lack of understanding of the molecular pathophysiology of schizophrenia, its diagnosis is based on its behavioral symptomatology. Unfortunately, due to only phenomenological diagnoses, these categories are inadequate. Therefore, we are examining human tissue for molecular causes and correlates of the illness. Our lab has proposed a model of psychosis as a disorder of learning and memory. We suggest that reduced glutamatergic neurotransmission to hippocampal CA3 serves to generate an increase in CA3 basal activity and function through homeostatic plasticity changes within CA3. This increase in function may lead to the generation of inappropriate or illogical memories with psychotic content. Our lab has shown an increase in perfusion in CA3 in schizophrenia, a correlate of regional neuronal activity level. Further supporting the notion of increased activity in CA3, we have also shown an increase in spine density and dendritic complexity in CA3 as well as increased protein levels of hypothesized candidate postsynaptic molecular markers like

GluN2B and PSD-95 in CA3 of schizophrenia postmortem tissue. We are in the process of further localizing these changes using immunohistochemistry. This will help elucidate which of the different afferent projections within CA3 are involved in this increased activity level. Importantly, we are analyzing CA3 subfield transcriptome from control and schizophrenia cases, in a global and unbiased manner, using whole transcriptome (WT) sequencing to identify additional molecular changes which have not been hypothesized. We have high quality CA3 tissue from human schizophrenia and control cases, who are not being treated with antipsychotic medication. The RNA is isolated and currently being sequenced to identify alterations associated with CA3 hyperperfusion.

Disclosures: J.M. Perez: None. **K. Gleason:** None. **S. Ghose:** None. **T. Kim:** None. **C. Tamminga:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.04/FF7

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH094670

Howard Hughes Medical Institute

Title: Hypo-NMDA receptor function leads to altered transcription in frontal cortex

Authors: *N. D. JOHNSON[1], C. A. PUDDIFOOT[2], J. R. NERY[2], M. URICH[2], E. A. MUKAMEL[2], R. LISTER[3], J. R. ECKER[2,4], T. J. SEJNOWSKI[2,4], M. M. BEHRENS[2]; [1]The Salk Inst., La Jolla, CA; [2]The Salk Inst. for Biol. Sci., La Jolla, CA; [3]Genomic Analysis Laboratory, Howard Hughes Med. Inst., The Salk Institute, La Jolla, CA; [4]Howard Hughes Med. Inst., Salk Institute, La Jolla, CA

Abstract: We have previously shown that prolonged N-Methyl-D-Aspartate receptor (NMDAR) blockade during a critical period for the development of parvalbumin-positive (PV+) interneurons, leads to altered electrophysiological properties and reduced PV+ cells in adult animals, resembling alterations observed in schizophrenia models. In this study, we use this NMDAR hypofunction mouse model to monitor transcriptome changes throughout development into early adulthood. mRNA was extracted from the frontal cortex of healthy and ketamine-treated mice at 2, 6, and 10 weeks of age, in order to study the genome-wide transcriptional changes that lead to altered PV(-) phenotype. Whole genome transcript expression was obtained

using Illumina 2000 RNAseq methods. Reads were aligned to the NCBI-37 reference genome using Bowtie and Tophat, and transcript quantification was performed using Cufflinks. For differential expression at each time-point we used both Cuffdiff and EdgeR computation analyses. Finally, to uncover functional groups of related genes we employed the Ingenuity IPA system.
This analysis revealed that at P13, two days after the last ketamine injection, 69 genes remained differentially-expressed as compared to the controls. Cuffdiff and EdgeR had a strong overlap in differential expression output between saline and ketamine samples. Gene ontology mapping identified that ketamine treated mice had altered expression of gene families involved in cell-cell signaling and neural cell development, consistent with an altered trajectory of cell development which may contribute to the phenotype observed in adolescent mice. Surprisingly, we have not observed changes in parvalbumin mRNA at any time point analyzed, suggesting decreased PV immunoreactivity after ketamine may be due to post-transcriptional changes. Cell-type specificity of these transcriptional changes is being assessed using immunohistochemistry, in situ hybridization and cell-type specific quantitative PCR.

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Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Program#/Poster#: 254.05/FF8

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: MH53327

Title: Dysregulation of gene expression in a glycosylation pathway in schizophrenia

Authors: A. HELIX[1], M. SIMMONS[1], T. MUELLER[1], V. HAROUTUNIAN[2], *J. H. MEADOR-WOODRUFF[1];
[1]Dept. of Psychiatry, Univ. Alabama at Birmingham, BIRMINGHAM, AL; [2]Psychiatry, Mt. Sinai Sch. of Med., New York, NY

Abstract: Previous findings from our lab have shown alterations of N-linked glycosylation of ionotropic glutamate receptor subunits, glutamate transcripts, and most recently GABA receptor subunits in cortical regions from postmortem brains of patients with schizophrenia (SZ). N-glycosylation is the process of enzymatically adding sugars to proteins which can facilitate their

transport through the endoplasmic reticulum and Golgi. Although our work to date has focused on determining alterations in N-glycosylation in candidate proteins associated with glutamate and GABA neurotransmission, we hypothesized that there may be widespread abnormalities of glycosylation. Accordingly, this study focused on measuring the expression of genes involved in glycosylation pathways in pyramidal neurons derived from prefrontal cortex. A human glycosylation pathway PCR array was used to measure changes of 84 genes associated with encoding enzymes that add and remove sugar residues to and from proteoglycans and glycoproteins. RNA was extracted and cDNA generated from 1000 laser capture microdissected pyramidal neurons from the DLPFC of 12 patients with schizophrenia and 12 matched comparison subjects. We identified 28 genes 1.37 fold or more in SZ which clustered into 14 different functional categories associated with glycosylation. N-acetylgalactosaminyltransferases, fucosidases, mannosidases, and enzymes associated with O-linked glycosylation were among those identified. These findings contribute to the growing body of evidence that supports our hypothesis that is glycosylation is widely dysregulated in schizophrenia. We are currently extending this work to determine if these changes are specific to pyramidal neurons or are found in other cells of the prefrontal cortex.

Disclosures: A. Helix: None. **J.H. Meador-Woodruff:** None. **V. Haroutunian:** None. **M. Simmons:** None. **T. Mueller:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.06/FF9

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: 5RC1MH088752-02

Title: Abnormal gene expression in striatal medium spiny neurons in schizophrenia

Authors: *M. S. SIMMONS[1], S. D. YATES[1], V. HAROUTUNIAN[2], R. MCCULLUMSMITH[1], J. H. MEADOR-WOODRUFF[1];
[1]PSYCH-BEHAVIORAL NEUROBIOGY, UAB, Birmingham, AL; [2]Psychiatry, Mt. Sinai Sch. of Med., New York, NY

Abstract: Schizophrenia (SZ) is a debilitating psychiatric illness with a not well understood pathophysiology. This complicated illness affects the whole brain and while many studies in postmortem brain have been published, a robust and reproducible finding has remained elusive. New and innovate tools allow the study of gene expression in defined cellular subpopulations

which may result in more specific studies in this illness. In this study, laser capture microdissection (LCM) was used to harvest medium spiny neurons in striatum from 12 pairs of subjects with schizophrenia and a matched control (Ctrl) using Affymetrix GeneChip® microarrays. Genes found to be changed in the microarray study were validated using qPCR. Genes that were altered were functionally categorized using Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7. Nucleus, transcription regulation, and plexin were among the highest scoring functional categories for genes that were changed between SZ and Ctrl in medium spiny neurons in striatum. Previous studies in our lab have been successful in finding gene alterations in other brain regions using this technique. These data add to those findings of changes in gene expression in defined cellular subpopulations of regions of the brain involved in the limbic system. These data also suggest novel pathways and potential targets disrupted in specific neuronal subpopulation in the striatum in SZ.

Disclosures: M.S. Simmons: None. **S.D. Yates:** None. **V. Haroutunian:** None. **R. McCullumsmith:** None. **J.H. Meador-Woodruff:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.07/FF10

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Sequential isolation of endosome subtypes from postmortem brain

Authors: *T. M. MUELLER, J. H. MEADOR-WOODRUFF;
UAB, Birmingham, AL

Abstract: Several studies have shown alterations in the expression of proteins associated with receptor trafficking and stability in schizophrenia. Previous work from our lab has shown alterations in the subcellular localization of AMPA receptor proteins as well as aberrant gene and protein expression of molecules associated with the trafficking of both NMDA and AMPA receptors. Alterations of N-glycosylation, which is known to play a functional role in protein trafficking, have also been identified for some GABAA and glutamate receptor subunits, supporting the hypothesis that mechanisms associated with the proper trafficking and subcellular localization of signaling molecules may be deficient in schizophrenia.
In order to address this question, we are developing a method to isolate multiple specific subcellular compartments from postmortem human brain. We have previously developed techniques to separately isolate intracellular vesicles necessary for the endocytosis, sorting, and

degradation of transmembrane proteins. Our isolations of both early and late endosomes, which respectively facilitate the sorting and targeted degradation of proteins, have been validated by both western blot and electron microscopy; however, these methods require a relatively large amount of starting material, costly substrates, and multiple time-consuming processing steps. We are seeking to improve the existing method to increase the efficiency of the isolations as well as to generate more data from limited amounts of sample by isolating multiple endosomal subtypes from the same starting material using sequential isolations. Additionally, we are working to develop an effective method to specifically isolate a third endosomal subtype, recycling endosomes, from postmortem human brain which we will validate by western blot and electron microscopy.
Ultimately, we will use these techniques to measure relative abundance of GABAA receptor subunits between early, late, and recycling endosomes from the same sample to provide a more accurate picture of how these subunits are being trafficked within the cell and identify alterations to the relative abundance of subunits in each endosomal compartment in schizophrenia. This will allow us to further elucidate whether aberrant GABAergic neurotransmission in schizophrenia may be attributed to or exacerbated by functional deficits of the endocytic pathway.

Disclosures: T.M. Mueller: None. **J.H. Meador-Woodruff:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.08/GG1

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: MH094445

Title: Altered neuregulin-1 intracellular domain dependent nuclear signaling in schizophrenia

Authors: D. SHAN[1], J. H. MEADOR-WOODRUFF[1], C. WALSS-BASS[2], *R. E. MCCULLUMSMITH[1];
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Abstract: Neuregulin-1(NRG1) regulates many important brain functions including synapse formation and synaptic transmission. NRG1 is a transmembrane protein cleaved into extracellular and intracellular domains (NRG-ICD) by enzymes in response to a variety of stimuli. Upon cleavage, NRG-ICD may translocate to the nucleus, where it regulates glutamateric gene expression. In the present study, we hypothesize that NRG-ICD dependent

nuclear signaling may be altered in schizophrenia attributable to changes in expression of NRG-ICD and gamma secretase which generates the intracellular domain, or disruption of NRG-ICD associated proteins, leading to abnormal glutamate levels in the synaptic cleft. We detected NRG-ICD protein in the prefrontal cortex in both control and schizophrenia subjects by Western blot analysis. Using fractionation, we found NRG-ICD is expressed in cytosol and nuclear fractions, indicating a nuclear translocation of NRG-ICD in the brain. We will also perform immunoisolation and mass spectrometry to identify NRG-ICD associated proteins. Our findings will identify the NRG-ICD associated proteins in different subcellular compartments, yielding novel candidates for probing the effects of NRG1 dysfunction in schizophrenia.

Disclosures: D. Shan: None. **R.E. McCullumsmith:** None. **J.H. Meador-Woodruff:** None. **C. Walss-Bass:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

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Program#/Poster#: 254.09/GG2

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH53327

NIH Grant MH066392

Title: Expression of AMPA receptors and their auxiliary proteins, TARPs, in isolated endoplasmic reticulum and postsynaptic density fractions from postmortem brain in schizophrenia

Authors: *J. B. DRUMMOND[1], V. HAROUTUNIAN[2], R. E. MCCULLUMSMITH[1], J. H. MEADOR-WOODRUFF[1];
[1]Psychiatry & Behavior Neurobio., Univ. Alabama, Birmingham, Birmingham, AL; [2]Psychiatry, Mount Sinai Hosp., Bronx, NY

Abstract: The glutamate hypothesis of schizophrenia suggests abnormal glutamatergic neurotransmission occurs in this illness. Recent evidence from our laboratory supports a model of altered forward trafficking and accelerated endoplasmic reticulum (ER) exit of the AMPA subtype of glutamate receptor (AMPAR) in schizophrenia. One mechanism that could lead to altered AMPAR trafficking is abnormal expression and localization of AMPAR auxiliary proteins, such as the transmembrane AMPAR regulatory proteins (TARPs). TARPs coassemble with AMPARs in the ER and traffic these receptors from the ER and Golgi apparatus to the

extrasynaptic membrane before mediating their lateral translocation to, and biophysical properties at, the postsynaptic density (PSD). Thus, TARP dysregulation may ultimately alter the expression, localization, stability and activity of AMPARs within intracellular compartments, potentially having a role in the pathophysiology of schizophrenia. We have previously reported changes in TARPs at the transcript and protein levels in homogenates of anterior cingulate cortex (ACC) in schizophrenia, suggesting altered intracellular trafficking of AMPARs in this illness. A model to explore AMPAR trafficking in postmortem brain involves examining specific, subcellular localization of these receptors and auxiliary proteins within compartments critical to AMPAR regulation. We hypothesized that these proteins are diminished in the ER in schizophrenia, consistent with our trafficking model. Utilizing a subcellular fractionation protocol optimized for human postmortem brain tissue, we measured TARP and AMPAR subunits within isolated ER from ACC in patients with schizophrenia and a comparison group. We found decreased GluA1 protein expression in the ER fraction in schizophrenia. We have recently validated a fraction enriched for isolated PSDs, and predict a subsequent reduction of GluA1 protein within this compartment consistent with our previous finding of increased GluA1 protein in an endosomal fraction in schizophrenia. Alterations in the expression and localization of AMPARs may present a new potential mechanism explaining glutamatergic dysregulation in schizophrenia.

Disclosures: J.B. Drummond: None. **V. Haroutunian:** None. **R.E. McCullumsmith:** None. **J.H. Meador-Woodruff:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

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Program#/Poster#: 254.10/GG3

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH53327

NIH Grant MH064673

NIH Grant MH066392

Title: Glycoproteome of the schizophrenia brain

Authors: *J. TUCHOLSKI[1], V. HAROUTUNIAN[2], J. H. MEADOR-WOODRUFF[1];
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[2]Psychiatry, Mount Sinai Sch. of Med., New York, NY

Abstract: Protein glycosylation regulates a wide range of processes critical to development and functioning of the central nervous system; these include cell adhesion, migration, neuronal and glial differentiation as well as synaptogenesis, synaptic function and plasticity. The majority of brain glycoproteins contain N-linked glycans (glycoconjugate) that are attached to an Asn residue within the consensus peptides sequence Asn-X-Ser/Thr (X ≠ Pro). A second class of glycoproteins contains O-linked glycans attached to the polypeptide via N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc) to a hydroxyl group of a Ser or Thr residue. We have previously observed an abnormal glycosylation of proteins in schizophrenia associated with the glutamate neurotransmitter system including EAAT1, EAAT2, GluA2, and GluK2, and the GABA system including $GABA_AR\alpha1$ and $GABA_AR\beta1$. This suggests that abnormalities in glycosylation may be involved in modulation of neuronal transmission or cell signaling events associated with schizophrenia pathophysiology. In this study, we investigated the glycosylation status of proteins in postmortem DLPFC of schizophrenia and comparison subjects using a lectin blotting approach with four different lectins: ConA recognizing glucose, or mannoses (2 or more); NPL recognizing mannose (prefers 2 or more); WGA, recognizing GlcNAc (2 or more); and SNA recognizing sialic acid attached to terminal Gal. We also identified glycoproteins altered in schizophrenia using nanoscale liquid chromatography-tandem mass spectrometry (nano LC-MS/MS). Findings from this study provide insights of identification of critical processes that are glycosylation-dependent and play a role in schizophrenia.

Disclosures: J. Tucholski: None. **V. Haroutunian:** None. **J.H. Meador-Woodruff:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

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Program#/Poster#: 254.11/GG4

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIH Grant K23 MH076976

NIH Grant R01 MH075895

Title: Neural synchrony patterns and levels of GABA and glutamate/glutamine during working memory task in schizophrenia

Authors: *R. SO[1], F. STEFFEN-ALLEN[1], L. KEGELES[2], J. J. CHROBAK[1], C.-M. A. CHEN[1]; [1]Psychology, Univ. of Connecticut, Storrs, CT; [2]Psychiatry and Radiology, Columbia Univ., New York, NY

Abstract: Synchronization of neural activity with the dorsolateral prefrontal cortex (DLPFC) is thought to be related to the neural mechanisms underlying cognitive functions, including working memory. Understanding the neural basis of cognitive deficits in schizophrenia would inform the development of new targeted interventions. The present study aims to examine the synchronization patterns between ipsilateral and contralateral hemispheres that differentiate individuals with schizophrenia and healthy controls. Scalp EEG recordings were collected for patients with schizophrenia (N = 7) and healthy volunteers (N = 8) during a modified Sternberg working memory task. Each trial consisted of three stages: 1) encoding stage, an array of one of two possible set sizes (one or six upper case letters) was presented on a computer screen for 3 seconds; 2) retention stage, a blank screen was presented for 7 seconds, and 3) probe stage, a test stimulus appeared for 3 seconds at the center of the screen. Participants were asked to identify whether the probe letter matches one of the letters in the array previously presented and then to respond by a button press as quickly and accurately as possible. For each session, each set size condition had 64 trials (total = 128 trials). Only correct, artifact-free trials were analyzed. Instantaneous phases were extracted by Morlet wavelet decomposition on 89 scales from 0.5 Hz to 60 Hz. Phase locking values (PLVs; ranging from 0 [no synchronization] to 1 [perfect synchronization]) indicate synchronization of neural activity between two selected electrodes. MRS measurements of both gamma-aminobutyric acid (GABA) and glutamate/glutamine (Glx) spectra were obtained. Routine measurements in the left DLPFC and anterior cingulated cortex were made on a 3T GE MR system, using the standard volume-selective PRESS J-editing difference method with a commercial 8-channel phased-array head coil. We test the hypotheses that 1) left DLPFC electrodes will have greater PLVs with their ipsilateral electrodes than with contralateral electrodes in the temporal, parietal, and occipital lobes; 2) left DLPFC electrodes will have greater PLVs with right DLPFC electrodes than contralateral electrodes in all other lobes; 3) strength and spatial patterns of PLVs from hypotheses 1 and 2 will differ between healthy controls and patients; 4) synchronization patterns will differ depending on working memory stage, and 5) synchronization patterns will correlate with GABA and Glx levels of MRS measures.

Disclosures: R. So: None. **F. Steffen-Allen:** None. **L. Kegeles:** 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.; Amgen. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Pfizer. **J.J. Chrobak:** None. **C.A. Chen:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIH Grant K23 MH076976

NIH Grant R01 MH075895

Title: Working memory performance, oscillatory patterns and levels of GABA and glutamine/glutamate in dorsolateral prefrontal cortex and anterior cingulate cortex in schizophrenia

Authors: *F. STEFFEN-ALLEN[1], R. P. SO[1], L. S. KEGELES[2], J. J. CHROBAK[1], C.-M. A. CHEN[1];
[1]Psychology, Univ. of Connecticut, Storrs, CT; [2]Psychiatry and Radiology, Columbia Univ., New York, NY

Abstract: One of the core features of schizophrenia is impairment in cognitive functions, which depend in part on the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC). The present study aims to investigate the possible linkage of working memory deficits in schizophrenia with changes in neuronal oscillations and prefrontal GABAergic dysfunction by relating EEG to magnetic resonance spectroscopy (MRS). Scalp EEG recordings were collected for patients with schizophrenia (N = 7) and healthy volunteers (N = 8) during a modified Sternberg working memory task. Each trial consisted of three stages: 1) encoding stage, an array of one of two possible set sizes (one or six uppercase letters) was presented on a computer screen for 3 seconds; 2) retention stage, a blank screen was presented for 7 seconds; 3) probe stage, a test stimulus appeared for 3 seconds at the center of the screen. Participants were asked to identify whether the probe letter matched one of the letters in the array previously presented and then to respond by a button press as quickly as possible. Each set size condition had 64 trials (total = 128 trials). Only correct, artifact-free trials were analyzed. MRS measurements of both gamma-aminobutyric acid (GABA) and glutamate/glutamine (Glx) spectra in left DLPFC and ACC were also obtained. “Efficiency index” for each participant was evaluated by testing the Pearson’s correlation coefficient between gamma power (30 to 58 Hz) and cued delayed-response time of single trials in each electrode. This “efficiency index” was then correlated with GABA levels, Glx levels, and phase synchronies of different frequency bands. In our sample, diagnosis did not predict working memory performance (p values > .05). However, preliminary results show that the “efficiency index” provides an alternative classification of participants that predicted cognitive performance. A Higher “efficiency index” indicates that as neural oscillatory activities increase, reaction times decrease. Participants with higher “efficiency indexes” had better performance on the working memory task. We test the hypotheses that the “efficiency index” correlates with: 1) GABA levels in left DLPFC and Glx levels in ACC, and 2) other oscillatory patterns.

Disclosures: F. Steffen-Allen: None. **R.P. So:** None. **L.S. Kegeles:** 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.; Amgen. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Pfizer. **C.A. Chen:** None. **J.J. Chrobak:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH076060

NIH Grant MH080272

Title: OTX2 expression in the human prefrontal cortex during normal postnatal development and in subjects with schizophrenia

Authors: *K. M. ATHANAS[1], S. A. MAUNEY[2], N. K. SHASKAN[2], T.-U. W. WOO[2,3,4]; [1]Lab. for Cell. and Mol. Neuropathology, [2]Lab. of Cell. and Mol. Neuropathology, McLean Hosp., Belmont, MA; [3]Psychiatry, Harvard Med. Sch., Boston, MA; [4]Psychiatry, Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: OTX2 is a homeoprotein that is involved in the regulation of many aspects of brain development across phylogeny. More recently, studies in rodents have shown that OTX2 regulates the postnatal maturation of parvalbumin (PV)-expressing interneurons and also drives the developmental formation of perineuronal nets (PNNs), which are extracellular matrix structures that encapsulate many neurons, including PV neurons. Of interest, PV neurons and PNNs are known to be compromised in schizophrenia (SZ). In this study, we investigate the developmental changes of OTX2 expression in the prefrontal cortex (PFC), focusing on the period of adolescence and young adulthood, and its possible alteration in SZ.
Using immunohistochemistry, we quantify OTX2-immunoreactive (IR) elements in postmortem tissue of Brodmann's area 9 of the PFC in a cohort of normal control human subjects (N=16), ages ranging from 2 days to 20 years old, and in a another cohort of 15 SZ subjects demographically matched with 15 normal control subjects. Qualitative examination reveals that OTX2-IR elements comprise pyramidal neurons, interneurons and spherical-like shaped structures that morphologically resemble corpora amylacea, although the definitive identity of these structures is unknown. Quantification of the densities of OTX2-IR elements in these two

cohorts of subjects is currently underway.
Findings of this study will provide insight into the possible role of OTX2 in normal PFC development during the periadolescent period, when SZ symptomatology typically begins to emerge. In addition, they will shed light onto how OTX2 may mediate the disturbances of PFC circuitry in SZ by compromising the integrity of PNNs and PV neuronal functions and, as such, may deepen our understanding of the possible mechanisms that underlie the onset and pathophysiology of SZ.

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Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Program#/Poster#: 254.14/GG7

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: R01MH0708551 A G

Title: DNA-Methylation gene network dysregulation in peripheral blood lymphocytes of schizophrenia patients

Authors: *J. AUTA[1], R. SMITH[2], E. DONG[3], P. TUETING[3], H. SERSHEN[2], S. BOULES[2], A. LAJTHA[2], J. DAVIS[3], A. GUIDOTTI[4];
[1]The Psychiatric Inst., Univ. of Illinois At Chicago, Chicago, IL; [2]Natl. S. Kline institute for Psychiatric Res., New York, NY; [4]Psychiatry, [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Recent studies suggest that epigenetic dysregulation of the brain genome that includes brain region-specific altered DNA promoter methylation is associated with the neuropathological manifestations of SZ and related psychiatric disorders (Guidotti et al., 2011; Grayson and Guidotti, 2013, Mill et al., 2008; Huston et al., 2013). The epigenetic dysregulation of the brain genome associated with the clinical manifestations of schizophrenia (SZ) includes altered DNA promoter methylation of several candidate genes. We and others have reported that two enzymes that belong to the DNA- methylation/demethylation network pathways- DNMT1 (DNA-methyltransferase) and TET1 (5-hydroxy cytosine translocator protein-1) are abnormally increased in corticolimbic structures of SZ postmortem brain. The objective of the study is to investigate whether the expression of the DNA-methylation/-demethylation network components known to be altered in discrete corticolimbic structures of SZ patients are also altered in peripheral blood lymphocytes (PBL). Peripheral blood lymphocytes (PBL) were isolated with

Ficoll-Paque Plus method. Total RNA was extracted using the TRIzol reagent (Life Technologies) and further purified using the Qiagen RNeasy Kit. Samples were assayed with qPCR using Fermentas Maxima SYBR Green/ROX qPCR Master Mix with primers designed to cross over one intron to amplify cDNA and yield an amplicon of between 75-200 base pairs. C_T value was used for relative quantification of target gene expression and normalized to β-actin and the relative expression levels were calculated as C_T. The data show that an increase in DNMT1 and TET1 and a decrease in glucocorticoid receptor (GCortR) and brain derived neurotrophic factor (BDNF) mRNAs in PBL of SZ patients are comparable to those reported in the brain of SZ patients. Thus these finding support the hypothesis that a common epigenetic dysregulation may be operative in the brain and peripheral tissues of SZ patients. If changes in these epigenetic biomarkers can be confirmed in lymphocytes of subjects with prodromal SZ syndrome before they progress to first episode SZ, some of the underlying molecular developmental pathology leading to SZ may be uncovered objectively in living subjects opening up the possibility of preventing onset of the disease.

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Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.15/GG8

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: Genome Quebec Grant

Title: Rare susceptibility variants for bipolar disorder: A family-based study

Authors: C. CRUCEANU[1,2], J. LOPEZ[1], A. AMBALAVANAN[1], P. A. DION[3], *N. MECHAWAR[2], M. ALDA[4], G. A. ROULEAU[3], G. TURECKI[2];
[1]Human Genet., McGill Univ., Montreal, QC, Canada; [2]Douglas Inst., Verdun, QC, Canada; [3]Montreal Neurolog. Inst., Montreal, QC, Canada; [4]Dalhousie Univ., Halifax, NS, Canada

Abstract: Bipolar disorder (BD) is a complex psychiatric condition characterized by both manic and depressive episodes. Previous studies strongly support the role of genetics in BD, with heritability estimates as high as 80%, but likely due to genetic and phenotypic heterogeneity, there has been minimal replication across studies. To address this problem we have been focusing on a well-defined sub-phenotype of BD, positive response to Lithium (Li) therapy, and shown that Li-response clusters in families. Research in BD genetics to date has consisted of

linkage and genome-wide association studies, which presume that common variants in a small subset of genes are the cause for BD. However, findings from these studies only explain a fraction of the predicted BD heritability, suggesting a causal role for highly penetrant rare variants in many different genes across the population. Our approach focuses on a well-defined clinical subtype of BD (Li-responsive) to minimize clinical heterogeneity, and we are using massively-parallel DNA sequencing to re-sequence the exomes of all affected individuals from multi-generational family units. To identify relevant BD susceptibility genes we are prioritizing rare variants that segregate with affected status within each family. To further explore the mechanisms by which these variants could lead to pathology we explore their expression in post-mortem brain samples and patient-specific lymphoblastoid cell lines. In each family we are prioritizing on average 12 potentially highly penetrant (e.g. protein-truncating, missense, or frameshift) or functionally relevant (e.g. 3'UTR, 5'UTR, splicing) variants. Some of the pathways that emerged from this analysis are involved in brain development and neurogenesis, inflammation, and epigenetic regulation - all processes that have been suggested to be dysregulated in mood disorders including BD. By focusing on rare variants in a familial cohort we hope to explain a significant portion of the missing heritability in bipolar disorder, as well as to have narrowed in on the key biochemical pathways that are implicated in this complex condition.

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Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Program#/Poster#: 254.16/GG9

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: MH071533

Title: Schizophrenia-associated alterations of microtubule associated protein 2 in human auditory cortex

Authors: *M. A. SHELTON[1], J. T. NEWMAN[1], K. N. FISH[1], P. PENZES[2], D. A. LEWIS[1], R. A. SWEET[1];
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Abstract: Background: The pathology of SZ includes laminar-specific reductions in dendritic spine density in the primary auditory cortex (PAC) and other cortical regions, suggesting functional alterations in the proteins responsible for maintenance of dendritic spine architecture. Microtubule-associated protein 2 (MAP2) is crucial for establishing and maintaining both dendrite and spine structure in response to changes in neural activity. A decrease in MAP2 immunoreactivity (IR) has been reported in several areas in SZ. We therefore hypothesized a role for MAP2 in reduced dendritic spine density in the PAC.
Methods: Using quantitative fluorescence microscopy coupled with immunohistochemical markers for dendritic spines and MAP2, we examined MAP2-IR and dendritic spine density in human post-mortem tissue taken from the PAC of individuals with SZ and matched controls.
Results: MAP2-IR based on an antibody targeting the protein's c-terminal microtubule binding domain was significantly ($p<0.05$) attenuated in SZ, with 60% of SZ subjects exhibiting fluorescence values near background, below the lowest values observed in controls. Reductions in dendritic spine density were restricted to those SZ subjects with reduced MAP2-IR. Restoration of MAP2-IR was achieved through antigen retrieval. As well, MAP2-IR was detected through the use of antibodies targeting alternate epitopes along the protein. This led us to hypothesize that the decrease in MAP2-IR is due to a disease specific post-translational effect that masks the c-terminal epitope targeted by our antibody.
Conclusions and Future Directions: Altered MAP2-IR is disease-associated and the changes are correlated with dendritic spine loss in these subjects. Using a combination of quantitative microscopy, antibody-based epitope mapping, and targeted proteomics, we will generate total and domain specific measures of MAP2 to determine how changes in protein levels and epitope availability contribute to the loss of MAP2-IR in SZ. Liquid chromatography-mass spectrometry will be used in an effort to elucidate post-translational modifications as well as atypical protein interactions which could mask recognition of the c-terminal epitope.

Disclosures: M.A. Shelton: None. **J.T. Newman:** None. **K.N. Fish:** None. **P. Penzes:** None. **D.A. Lewis:** 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.; Bristol-Meyers Squibb, Curridium Ltd and Pfizer. F. Consulting Fees (e.g., advisory boards); Bristol-Meyers Squibb, Concert Pharmaceuticals. **R.A. Sweet:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Topic: C.16. Schizophrenia and Bi-polar Disorder

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NIH R21 NS076958

NIH P50 MH096890

Title: Conserved chromosome 2q31 conformations are associated with transcriptional regulation of GABA synthesis enzyme GAD1 and altered in prefrontal cortex of subjects with schizophrenia

Authors: *Y. JIANG, S. AKBARIAN;
CSM building, 9th floor, room 202, Mount Sinai Sch. of Med., New York, NY

Abstract: Little is known about chromosomal loopings involving proximal promoter and distal enhancer elements regulating GABAergic gene expression, including changes in schizophrenia and other psychiatric conditions. Here, we map in human chromosome 2q31 the 3-dimensional configuration of 200 Kb of linear sequence encompassing the GABA synthesis enzyme gene GAD1 locus, and describe a loop formation involving the GAD1 transcription start site (TSS) and intergenic non-coding DNA elements (50Kb) facilitating reporter gene expression. The GAD1-TSS-50Kb Loop was enriched with nucleosomes epigenetically decorated with the transcriptional mark, histone H3 trimethylated at lysine 4 (H3K4me3), and was weak or absent in skin fibroblasts and pluripotent stem cells as compared to neuronal cultures differentiated from them. In primary neuronal culture, Gad1-TSS-55Kb Loop and Gad1 expression became upregulated when neuronal activity was increased. In the prefrontal cortex of subjects with schizophrenia, GAD1-TSS-50Kb Loop was decreased compared to controls, in conjunction with downregulated GAD1 expression. We generated transgenic mice expressing Gad2 promoter-driven green fluorescent protein-conjugated histone H2B and confirmed that Gad1-TSS-55Kb Loop, the murine homologue to GAD1-TSS-50Kb Loop, is a chromosomal conformation specific for GABAergic neuron. We conclude that 3-dimensional genome architectures, including chromosomal loopings for promoter-enhancer interactions involved in the regulation of GABAergic gene expression, are conserved between the rodent and primate brain, and subject to developmental and activity-dependent regulation, and disordered in some cases with schizophrenia. More broadly, the findings presented here draw a connection between non-coding DNA, spatial genome architecture, and neuronal plasticity in development and disease.

Disclosures: Y. Jiang: None. **S. Akbarian:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NARSAD/ Sidney R. Baer Jr. Foundation Young Investigator Award

Institute for Mental Health Research

Title: Case-control and family association study of early growth response gene 3 with schizophrenia

Authors: *A. L. GALLITANO[1], L. MUPPANA[2], J. CORNEVEAUX[3], A. M. AHMED[4], M. HUENTELMAN[3];
[1]Basic Med. Sci., Univ. Arizona COM PHX, PHOENIX, AZ; [2]Basic Med. Sci., Univ. of Arizona Col. of Med. - Phoenix, Phoenix, AZ; [3]Translational Genomics Res. Inst., Phoenix, AZ; [4]Arizona State Univ., Tempe, AZ

Abstract: A major obstacle in identifying genes that influence risk for psychiatric disorders is that these illnesses are determined by both genetic and environmental factors. Assessments of the genetic contribution to the risk of developing schizophrenia, one of the most severe of the mental illnesses, range from 50-80%. Environment accounts for the remaining influence. In the current study we have addressed this issue by examining a family of immediate early genes that are activated in response to environmental stimuli, such as stress, and in turn regulate the expression of genes involved in numerous biological processes that are disrupted in schizophrenia. The Early Growth Response (EGR) family of genes includes four members, EGR1, EGR2, EGR3, and EGR4, which encode transcription factors. Studies in the Japanese, Korean, and Han Chinese populations have reported significant associations between single nucleotide polymorphisms (SNPs) in EGR3 and schizophrenia. In the current study we used a case-control analysis to examine whether SNPs in EGRs 1- 4 were associated with schizophrenia. We then employed a novel Next-Generation Sequencing approach to screen the EGR3 locus for SNPs that varied in minor allele frequency between pooled samples of cases and controls in two racial populations. This analysis allowed rapid identification of promising SNPs, one of which was selected for follow-up genotyping in a larger cohort. In addition, we performed a family association study examining a SNP in EGR3 for preferential inheritance in a group of 244 family trios. We will present findings showing that a SNP in the EGR3 gene was preferentially inherited by probands with schizophrenia. Case-control studies revealed a nominally significant association between the minor allele of this SNP and schizophrenia in the white population that did not survive Bonferroni correction for multiple comparisons. No significant difference in the prevalence of this SNP was found in the black cohort. SNPs in the EGR1, EGR2, and EGR4 genes did not display any significant associations with schizophrenia in this study.

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Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: IMSD, CESCOM, FAU

EUGEI

Title: Convergent in silico analysis of genes for interneurons and hypoxia response under the neurodevelopmental hypothesis of schizophrenia

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Abstract: We have proposed that schizophrenia (SCZ) candidate genes may interact with hypoxia in neurodevelopment under a gene x environment interaction model (Schmidt-Kastner et al., Mol. Psychiatry 2012). Neuroanatomical and functional studies suggest an impairment of GABAergic, cortical interneurons in the manifest stage of SCZ. Decreased cortical inhibition may be related to the NMDA-receptor hypofunction hypothesis of SCZ (Olney et al., J. Psychiatr. Res. 1999) which gained fresh support from exome sequencing of GRM5 (Timms et al., JAMA Psychiatry 2013). We here suggest that interneurons may be particularly vulnerable to metabolic and other environmental stresses, including hypoxia and ethanol, during or after their complex, tangential migration from the ganglionic eminence to the cortex. This data mining study examines convergence between SCZ candidate genes, genes related to developing interneurons and hypoxia response. Candidate genes for SCZ were retrieved from the SzGene database (n=360). Genes reported to be involved in the neurodevelopment of cortical interneurons (INTDEV; n=416) were compiled from literature, based on transgenic mice with interneuron-specific reporters, cell sorting and gene expression profiling. To annotate INTDEV, we used our databases for ischemia-hypoxia response (IHR) genes, HIF-1 regulated genes, and vascular genes. Databases for copy number variations (CNVs) in neurodevelopmental delay were

studied for links to INTDEV. In total, n=147 (35%) of INTDEV genes were annotated with our databases. Thereby, n=94 INTDEV genes matched with the IHR gene database (22% observed vs. 9% random; chi-square test, p = 0.0001). Enrichment among INTDEV genes was found for vascular genes (x2.3), but not for astrocytic genes (x1.3). N=29 (7%) of SCZ candidate genes were on the INTDEV list. N=12 genes were found in the convergent analysis [SCZ x INTDEV x IHR genes], including CACNA1C, CCKAR, DLX1, GRM5 and NPY. Using data mining of CNVs and protein interactions, a novel link from haploinsufficient, hypoxia-responsive genes to glutamate receptor function, including GRM5, was developed. In conclusion, we suggest than an early hypoxic hit during critical phases of tangential migration could impair cortical interneurons, inducing long-lasting glutamate receptor dysfunction. Our convergent analysis proposes genes related to both development of interneurons (assuming specificity of the selection process) and hypoxia response for future studies, including epigenetics. The overlap between vascular genes and INTDEV genes supports recent reports of pleiotropic gene function.

Disclosures: R. Schmidt-Kastner: None. **M. Joseph:** None. **K. McKain:** None. **J.W. Newcomer:** None. **J. van Os:** None. **B.P.F. Rutten:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.20/GG13

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: 2011 NARSAD young investigator award

NIH R01 grant MH085666

Title: Disruption of AKT alters dopamine sensitivity to inhibitory synaptic transmission in the rat prefrontal cortex

Authors: *Y. LI, W.-J. GAO;
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Abstract: AKT, also known as PKB, is a serine/threonine kinase that has been found dramatically reduced in the prefrontal cortex (PFC) of patients with schizophrenia. AKT1 deficiency causes abnormalities in the PFC
function, particularly working memory function in the AKT mutant mice. However, how AKT deficiency affects the PFC function is not well studied. Dopamine (DA) as a major neurotransmitter in the brain plays an important role in normal prefrontal function and

neuropsychiatric disorders. Although AKT is known important for dopaminergic transmission to maintain normal function of PFC, whether and how impairment of AKT affects dopaminergic modulation of synaptic transmission in the PFC is not clearly understood. In this study, we try to mimic AKT deficiency by bath application of AKT inhibitors in the in vitro whole-cell clamp recording of inhibitory postsynaptic currents (IPSC) in the rat PFC and then examine the DA modulation. We found that either bath application of 10 μM 10-DEBC which is a permeable AKT inhibitor or 5 μM AKT inhibitor VI , an impermeable AKT inhibitor, significantly shifted dopaminergic modulation of GABAa receptor-mediated IPSCs in PFC layer V pyramidal neurons, suggesting an reduced sensitivity to DA. The desensitization of DA to inhibitory transmission caused by inhibition of AKT is likely involved in both pre- and postsynaptic mechanisms although paired-pulse ratio was not altered. Interestingly, inhibition of AKT significantly disrupted both D1R- and D2R-mediated dopamine effects on IPSCs although it had stronger effects on D2R-mediated action. The effect of inhibiting AKT on DA modulation of evoked IPSCs, however, disappeared after inhibiting β-arrestin2, a protein integrator important for G protein-coupled receptor desensitization. Consistently, the protein level β-arrestin2 was significantly increased whereas the protein level of D2 receptors on synaptic membranes was significantly decreased by co-application of AKT inhibitor and DA in the prefrontal cortical slices. Take together, our data suggest that that AKT deficiency leads to reduction of DA sensitivity to inhibitory synaptic transmission through β-arrestin2 dependent DA receptor desensitization.

Disclosures: Y. Li: None. **W. Gao:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NSERC Grant No. 355923

FRSQ Grant No. 16193

Title: Chronic haloperidol enhances amphetamine-induced conditioned reward: Contributions of the dorsal and ventral striatum

Authors: *C. EL HAGE[1], J. GUILLEMETTE LAFONTAINE[1], A.-M. BÉDARD[2], A.-N. SAMAHA[1];

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Abstract: Drug abuse and addiction are excessively common in schizophrenia. It has been proposed that chronic exposure to antipsychotics might contribute to this co-morbidity by altering the reward system. Indeed, we have shown previously that rats withdrawn from continuous haloperidol (HAL) treatment (via a sub-cutaneous minipump) pursue reward cues more vigorously than HAL-naive rats following an intra-peritoneal amphetamine (AMPH) injection. AMPH-induced potentiation of conditioned reward is mediated in great part by the drug's actions in the nucleus accumbens (NAc), and to a lesser extent, in the caudate-putamen (CPu).

Our aim here was to determine the contributions of the NAc and CPu in the ability of HAL to augment AMPH-induced potentiation of conditioned reward. Rats were trained to associate a light-tone cue with water and then treated with a clinically relevant dose of HAL via osmotic minipump for 15 days. Following antipsychotic withdrawal, we assessed lever pressing for the light-tone cue (now a conditioned reward) after intra-NAc or intra-CPu AMPH injections (0 to 20 µg/hemisphere).

Across a range of doses and in both control and HAL rats, intra-CPu AMPH did not alter lever pressing for conditioned reward. In accordance with the literature, intra-NAc AMPH enhanced operant responding for the conditioned reward in control rats. However, there was no effect in the HAL-treated rats, at any AMPH dose tested. These findings suggest that continuous HAL modifies reward circuitry such that stimulation of the NAc with AMPH is no longer sufficient to increase the operant pursuit of reward cues. Our objectives now are two-fold: first, to determine whether the NAc remains necessary for the ability of HAL treatment to augment AMPH-induced potentiation of conditioned reward and second, to determine whether this effect of AMPH remains amenable to disruption by D1 and D2 receptor antagonists in HAL-treated rats as it is in control animals. The hope is that this work might shed new light on the neurobiological substrates by which antipsychotic treatment alters reward function.

Disclosures: C. El Hage: None. **J. Guillemette Lafontaine:** None. **A. Bédard:** None. **A. Samaha:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Support: NIH Grant MH080272

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Title: mRNA and miRNA expression profiling of pyramidal, parvalbumin-containing and dopamine neurons in schizophrenia and Parkinson's disease

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Abstract: The human brain is an extraordinarily complex structure consisting of heterogeneous subsets of neurons that mediate distinct aspects of information processing. Disturbances of these neurons compromise the functional integrity of the connectional architecture of the brain, resulting in various psychiatric and neurologic disorders. In order to explore how the molecular integrity of various neuronal subtypes might be compromised in schizophrenia (SZ) or Parkinson's disease (PD), we combined laser capture microdissection, microarray and TaqMan-based miRNA profiling technologies to determine the convergence and specificity of the gene networks and signaling cascades that are altered in these disorders. In pyramidal neurons from the prefrontal cortex in SZ, we found differentially expressed mRNAs that belong to the transforming growth factor beta and the bone morphogenetic proteins signaling pathways, and in the parvalbumin (PV)-immunolabeled neurons from the same region differentially expressed transcripts were associated with WNT, NOTCH and PGE2 signaling, in addition to genes that regulate cell cycle and apoptosis. In the dopamine neurons from the substantia nigra in PD, there was a predominant down-regulation of genes that are involved in PD pathogenesis, such as members of the PARK gene family and genes associated with programmed cell death, mitochondrial dysfunction, neurotransmitter and ion channel receptors, as well as neuronal survival mechanisms. In addition to the gene expression profiles, we identified a set of differentially expressed miRNAs in both SZ and PD. Enrichment analysis of their predicted targets revealed signaling pathways and gene networks that were also found by the microarrays to be dysregulated raising an interesting possibility that dysfunction of these neurons in SZ or PD may in part be mediated by a concerted dysregulation of gene network functions as a result of the altered expression of miRNAs. In conclusion, our data show mostly distinctive, but also some overlapping dysfunctional gene and miRNA networks between SZ and late stage PD, and provide a platform for future downstream analyses aiming to understand the molecular processes of individual neuronal dysfunction in psychiatric and neurological disorders.

Disclosures: T. Woo: None. **K. Sonntag:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

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Title: Abnormal phospholipids distribution in the postmortem brains from patients with schizophrenia revealed by matrix-assisted laser desorption/ionization imaging mass spectrometry: Usefulness of antemortem psychiatric clinical information in postmortem brain study

Authors: *J. MATSUMOTO[1], Y. KUNII[2], T. HAYASAKA[3], M. HINO[2], A. WADA[2], H. AKATSU[4], Y. HASHIZUME[4], T. YAMAMOTO[4], S. SATO[5], H. YABE[2], M. SETOU[3], S.-I. NIWA[2];
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Abstract: Background
Since lipid metabolism plays an important role in the functioning of the brain, the hypothesis that lipid abnormalities are involved in schizophrenia is important to clarify the molecular mechanism of schizophrenia. Nevertheless lipid analysis using postmortem brain samples is not enough. Also, in the previous psychiatric postmortem studies there were little ones which effectively used antemortem psychiatric clinical information, although most researchers performed case-control studies to detect disease specific alternations. Imaging mass spectrometry (IMS) can be used to more precisely analyze the postmortem brain samples from patients with schizophrenia. In this study, we have analyzed the results from imaging IMS utilizing antemortem psychiatric clinical information.
Methods
We performed IMS-based lipid analysis by using brain samples from 12 patients with schizophrenia and 10 control subjects. The postmortem brain tissues from patients with schizophrenia were obtained from the Postmortem Brain Bank of Fukushima for Psychiatric Research. We analyzed the gray and white matter of the frontal and temporal lobes, as these lobes tend to become atrophic in patients with schizophrenia. We examined the average intensity

of the phospholipid detected by IMS and we reanalyzed the above results using structured retrospective review of psychiatric records, DIBS (Diagnostic Instrument for Brain Studies) 1.
Results
The schizophrenia and control groups did not differ with respect to the average expression of phosphatidylcholine, sphingomyelin, galactosylceramide, phosphatidylethanolamine, phosphatidylserine, and sulfatide. The phosphatidylinositol (PI) content was decreased in patients with schizophrenia. We found that the psychiatric symptoms were severer in the group which indicated higher or lower intensity of the PI detected by mass spectrometry compared to the group which indicated middle intensity. These tendencies were also observed narrowing analysis down to the positive symptoms.
Conclusions
The decreased expression of PI observed in the prefrontal cortex of schizophrenia would indicate evidence of abnormality of phospholipid metabolism in the prefrontal cortex of schizophrenia. We also reanalyzed the results utilizing antemortem psychiatric symptoms and found some interesting results, which may lead to new significant findings in the relevant research areas.
1.Keks NA, Hill C, Roberts S et al. Diagnostic instrument for brain studies (DIBS). Schizophrenia Research 1997; 24: 34-34.

Disclosures: J. Matsumoto: None. **Y. Kunii:** None. **T. Hayasaka:** None. **M. Hino:** None. **A. Wada:** None. **H. Akatsu:** None. **Y. Hashizume:** None. **T. Yamamoto:** None. **S. Sato:** None. **H. Yabe:** None. **M. Setou:** None. **S. Niwa:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.24/GG17

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Is this really schizophrenia?

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Abstract: _
Introduction: A 34 year old man was referred to our unit for psychiatric evaluation, due to

suspected catatonic schizophrenia. His psychomotor development was normal until the age of 12 , when he started showing progressive loss of cognitive skills, fine motor skills and finalized motor activity. Symptoms rapidly worsened, and by the age of 20 his language and motor skills were severely compromised (he needed repeated verbal stimulation to initiate motor activity or speech, and could only sustain them for a few minutes). Starting at age 17 he had been treated with both 1st and 2nd generation antipsychotics, which did not lead to any changes in symptoms. Family history was negative for neurological disorders. His mother was diagnosed with alcohol abuse disorder and the younger sister was diagnosed with generalized anxiety disorder and borderline personality disorder.

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Method: At the time of our evaluation the patient was receiving 10 mg of Olanzapine. He was vigilant and passively cooperative to examination; he displayed physical immobility, echopraxia, waxy flexibility, stereotyped behaviour, camptocormia, echolalia, blunted affect and automatic obedience. Thought content was difficult to evaluate, but he denied hallucinations or delusional thoughts. Cranial nerves, strength, tone, reflexes were normal, there were no signs of cerebellar dysfunction, and the only abnormality noted on neurological exam was parkinsonian gait.

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Results: Blood panel was normal, as were thyroid and adrenal function, prolactin levels and B12. The patient was screened for Wilson's and hemochromatosis and no abnormalities were found. Sreening for metabolic disease was performed, suspecting Nieman Pick type C, and was also negative (normal aminoacidaemia and aminoaciduria). Genetic testing revealed a normal male cariotype and was negative for fragile X syndrome. Brain resting state MRI, PET scan, EEG and DAT scan were also performed and showed no abnormalities. Antipsychotic medication withdrawal didn't lead to any changes in his psychiatric or neurological exam. He was prescribed Lorazepam which didn't improve his symptoms.

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Conclusions: The patient's symtoms fulfill the criteria for Catatonic Schizophrenia. However the early onset of symptoms, the rapid deterioration of cognitive abilities, the early and extensive motor imparment and the complete resistance to drug treatment make this an interesting case for discussion about differential diagnosis. We are currently screening for Huntigton's Chorea.

Disclosures: L. Islam: None. **L. Romito:** None. **G. Tisi:** None. **M. Carboni:** None. **S. Scarone:** None. **O. Gambini:** None.

Poster

254. Schizophrenia: Molecular and Cellular Mechanisms

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIMH Grant 1K08MH087640-01A1

NIMH Grant 5 R01 MH51290-13

NIMH Grant MH077175

Title: Actin cytoskeleton dysregulation in schizophrenia and bipolar disorder: Relevance to dendritic spine pathology

Authors: *G. T. KONOPASKE, S. SUBBURAJU, J. T. COYLE, F. M. BENES;
McLean Hosp., Belmont, MA

Abstract: Schizophrenia is a severe and persistent mental illness affecting 1% of the population worldwide. Previously, dendritic spine density associated with pyramidal cells was found to be reduced in the deep half of layer III in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia. Since the actin cytoskeleton plays a central role in the development, maintenance, and function of dendritic spines, we sought to determine if dysregulation of the actin cytoskeleton might account for the observed dendritic spine pathology. By analyzing microarray data obtained previously using DLPFC tissue from subjects with schizophrenia (n=19), bipolar disorder (n=18), and unaffected control subjects (n=25), we identified 5 candidate genes (IFG1R, MARCKS, PPP1R9A, PTPRF, and ARHGEF2) that regulate both the actin cytoskeleton and the formation or maintenance of dendritic spines. Using a microarray-based analysis of gene expression, IFG1R, MARCKS, PPP1R9A, and PTPRF exhibited increased expression in schizophrenia subjects relative to controls. However, ARHGEF2 exhibited decreased expression in subjects with schizophrenia relative to controls. The expression of all five genes did not differ between the bipolar disorder and control subjects. The microarray data were validated using quantitative real-time PCR in a second cohort of subjects with schizophrenia (n=19), bipolar disorder (n=17), and unaffected control subjects (n=18). In the DLPFC, the expression of IGF1R and MARCKS was significantly increased in both the schizophrenia and bipolar disorder subjects relative to the controls. In addition, PPP1R9A expression was significantly increased in bipolar disorder subjects relative to both schizophrenia and unaffected control subjects. The expression of PPP1R9A did not differ between schizophrenia and control subjects. Moreover, PTPRF and ARHGEF2 expression levels did not differ among the groups. Post hoc analyses correlating gene expression with dendritic spine density measurements from the same subjects will also be presented. Overall, the regulation of the actin cytoskeleton and dendritic spines appears to be altered in both schizophrenia and bipolar disorder and may possibly reflect neuropathological changes in layer III of the DLPFC.

Disclosures: G.T. Konopaske: None. **S. Subburaju:** None. **J.T. Coyle:** F. Consulting Fees (e.g., advisory boards); ABBvie. **F.M. Benes:** 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.; Takeda.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.26/HH1

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: MH096985

DGE-0549352

MH051234

Title: Differential distribution of the GABA synthesizing enzymes in somatostatin containing boutons of the monkey prefrontal cortex-implications for schizophrenia

Authors: B. ROCCO, D. A. LEWIS, *K. FISH;
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Abstract: In the prefrontal cortex (PFC) of subjects with schizophrenia, the density of neurons detectable by GAD67 mRNA is reduced by ~30%. This deficit occurs without a change in total neuron number, suggesting that the affected GABAergic neurons are present but contain severe reductions in GAD67 expression. In contrast, mRNA expression for GAD65 is unchanged in schizophrenia. We recently showed in non-human primate PFC that boutons belonging to cannabinoid 1 receptor-expressing basket cells contained only GAD65, those of parvalbumin (PV)-expressing chandelier cells (PVChC) contained only GAD67, and PV basket cell boutons contained both GAD isoforms. Previous findings suggest that the density of PVChC boutons is reduced in schizophrenia, whereas the density of GAD65 boutons is unchanged, which led us to hypothesize that boutons containing only GAD67 are uniquely vulnerable to GAD67 deficits in the illness.
In schizophrenia, both the density of somatostatin (SST) mRNA-positive neurons in the PFC gray matter and the expression of SST mRNA per neuron are lower. In addition, lower expression of SST mRNA is positively correlated with lower levels of GAD67 at both the tissue and cellular levels. In order to determine the possible vulnerability of SST boutons to reduced GAD67 expression, we used quantitative fluorescence microscopy to measure their relative GAD65 and GAD67 content. We found that SST neurons give rise to boutons that contain only GAD65, only GAD67, or both GAD isoforms. Considering that the majority of SST boutons

contained only GAD67, we hypothesize that a significant reduction in GAD67 expression in SST neurons could result in a loss of SST boutons. As a first step in testing this hypothesis, the density of non-PV, GAD67 only boutons was assessed in PFC tissue sections from five matched pairs of schizophrenia and comparison subjects. In schizophrenia, the mean density of non-PV, GAD67 only boutons was significantly reduced 70% compared to control subjects ($P = 0.05$). In addition, the average volume and total GAD67 fluorescence intensity of the remaining non-PV, GAD67 only boutons were significantly less ($p = 0.02$ and 0.03, respectively). Considering that PVChC boutons were excluded from our assessment and a subsequent experiment found that the vast majority of boutons arising from calretinin expressing neurons contain GAD65, our results suggest that the density of SST boutons containing only GAD67 is significantly lower in schizophrenia. SST neurons are important for synaptic integration and deficits in SST neuron signaling may result in alterations in the proper input/output functions of neuronal ensembles in the PFC.

Disclosures: B. Rocco: None. **D.A. Lewis:** 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.; Bristol-Myers Squibb, Pfizer. F. Consulting Fees (e.g., advisory boards); Bristol-Myers Squibb, Concert Pharmaceuticals. **K. Fish:** None.

Poster

254. **Schizophrenia: Molecular and Cellular Mechanisms**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 254.27/HH2

Topic: D.02. Auditory

Title: The effect of reboxetine and haloperidol on P50 suppression in healthy volunteers

Authors: *L. M. WITTEN[1,2], B. Y. GLENTHØJ[2], A. MØRK[1], B. STEINIGER-BRACH[1], J. F. BASTLUND[1], B. ORANJE[2];
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Abstract: Introduction: Disruptions in filtering of sensory information have frequently been observed in patients with schizophrenia. Successful sensory gating prevents sensory overload of higher brain functions by filtering out irrelevant stimuli before they can reach the higher brain areas. Deficits in sensory gating may therefore result in an overload of irrelevant information

reaching the higher brain areas, which in turn might contribute to the formation of psychotic symptoms. One well established paradigm to assess sensory gating is P50 suppression. In this paradigm, patients with schizophrenia score significantly lower than healthy controls. In schizophrenia both a reduction in prefrontal dopaminergic activity and an increased noradrenergic activity have been suggested to be involved in the disease. In the current study we aimed to further investigate the involvement of these neurotransmitters using the norepinephrine reuptake inhibitor (NRI) reboxetine and the dopamine antagonist haloperidol, to increase noradrenergic as well as decrease dopaminergic neurotransmitter activity, respectively.
Material and methods: The design of the experiment was a double-blind, placebo-controlled, cross-over study, where a dose of either reboxetine (8 mg), haloperidol (2 mg), their combination or placebo was administered to 21 healthy male subjects at four separate visits with a minimum of two weeks apart. The subjects were subsequently tested in The Copenhagen Psychophysiological Test Battery (CPTB) which, amongst others, measures P50 suppression using electroencephalography (EEG).
Results: We found a significant reduction in P50 suppression following separate administration of either reboxetine or haloperidol as well as following their combined administration compared to placebo.
Conclusion: The current study aimed to help clarify the neurotransmitter systems involved in sensory gating. Based on the results obtained it appears that both an increased noradrenergic and a decreased DA activity is involved in P50 suppression. However we did not observe a synergistic effect of the combination of the compounds, which might indicate a ceiling effect or a drug/drug interaction. Since sensory gating in schizophrenia patients is usually found to be reduced compared to controls our results may indicate similar underlying neurotransmitter activity.

Disclosures: L.M. Witten: None. **B.Y. Glenthøj:** None. **A. Mørk:** None. **B. Steiniger-Brach:** None. **J.F. Bastlund:** None. **B. Oranje:** None.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.01/HH3

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: An orthosteric mGluR 2/3 Agonist, but not a Positive Allosteric Modulator, enhances acoustic startle response in rodents

Authors: *R. WILLEMS, L. VER DONCK;
Neurosci., Janssen Res. & Development, A Div. of Janssen Pharmaceutica NV, Beerse, Belgium

Abstract: The acoustic startle response (ASR) in rodents is a useful model to study the plasticity of sensorimotor information processing in mammals, including habituation and sensitization of startle and prepulse inhibition. The ASR is a relatively simple response characterized by a rapid contraction of skeletal muscles following an unexpected and intense acoustic stimulus. Changes in startle amplitude through repeated stimulus presentation or pharmacological intervention represent the simplest form of learning and have been used to identify the neural basis of learning in invertebrates. Since glutamate appears to be a prominent neurotransmitter in the auditory system, modulation of its receptors might impact the ASR.
OBJECTIVE: To compare the orthosteric mGluR2/3 agonist LY-404039 (LY) and a novel selective mGluR2 Positive Allosteric Modulator (JNJ-PAM, JNJ-40411813) on modulation of ASR.
METHODS: Male mice (NMRI and C57BL6J (BL6)) and rats (Wistar (WI) and Sprague Dawley (SD)) were treated with LY, PAM or vehicle, thirty min before testing. ASR was measured using the Acoustic Startle Reflex System (San Diego Instruments, US) producing a random mix of white noise sound pulses (70-120 dB vs. 65 dB background, pulse length: 40ms).
RESULTS: In a series of independent studies in NMRI mice, the mGluR2/3 agonist LY increased ASR at 2.5 and 10 mg/kg compared to control, from 90 dB pulse intensity onwards, ($p<0.05$ and $p<=0.001$ respectively, RM-ANOVA) whereas JNJ-PAM was without effect. In contrast, the LY-induced increase in ASR was not observed in BL6 mice. The reason for this strain difference is unclear. Increased responses were also measured in both rat strains with LY at 10 mg/kg ($p<0.001$) while no effects were found with JNJ-PAM.
CONCLUSION:
1/ Increased ASR was demonstrated in rodents by the orthosteric mGluR2/3 agonist LY-404039 but not by the mGluR2 PAM JNJ-40411813. This might indicate that modulation of the ASR is driven by mGluR3 stimulation.
2/ Continued receptor activation by an agonist may over stimulate the system, while a PAM avoids this by only modulating in the presence of physiologically released glutamate.
3/ Effects of an orthosteric mGluR2/3 agonist in behavioural tests involving startle response should be interpreted with caution.

Disclosures: R. Willems: A. Employment/Salary (full or part-time):; Janssen Reseach&Developmen, A division of Janssen Pharmaceutica NV. **L. Ver Donck:** A. Employment/Salary (full or part-time):; Janssen Reseach&Developmen, A division of Janssen Pharmaceutica NV.

Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

Location: Halls B-H

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Program#/Poster#: 255.02/HH4

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NSERC Discovery Grant 402642

Title: Chronic ketamine administration induces enhanced mismatch and novelty detection in rats

Authors: *A. SCHUMACHER, E. C. TOLLEDO, L. FERGUSON, R. ITO;
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Abstract: Chronic ketamine administration has been widely used as an animal model of schizophrenia. Furthermore, some recent work indicates that it may has superior face validity as it can induce both positive-, as well as negative-like symptoms, and cognitive deficits in rats. This is most likely due to the fact that ketamine's primary mechanism of action is upon the glutamatergic system, and yet it can also induce alterations in the mesocorticolimbic dopamine system. The present study investigated the effect of repeated ketamine pre-treatment upon novelty detection, which is associated with reduced hippocampal recruitment in schizophrenics, and other indices of negative symptoms of schizophrenia such as anhedonia and locomotor activity. Male Long Evans rats received repeated intra-peritoneal injections of ketamine or saline. After a withdrawal period of 10 days, rats were tested in an associative mismatch detection task to examine their ability of detecting familiar audiovisual stimuli appearing in novel configurations. Rats were exposed to two audiovisual sequences over four training days, and exhibited an orienting response (OR) to the visual (light) stimulus as a behavioral index of novelty detection, which habituated across the training days. On the final test day, the two audiovisual pairings were scrambled, such that rats were presented with novel stimulus configurations, alongside familiar stimulus configurations. Furthermore, rats underwent a novel object detection task. Rats were habituated to four objects presented in each arm of a plus maze. Subsequently, one of the 4 objects was replaced by a new object, and the spatial locations of two familiar objects were switched. Additionally, rats underwent a sucrose preference test, and locomotor tests involving administering a challenge dose of amphetamine. Ketamine pre-treated rats showed significantly better mismatch detection and an enhanced ability of distinguishing spatially novel objects compared to saline rats. Furthermore, ketamine-treated rats exhibited a state of heightened hedonia, and higher spontaneous locomotor activity than saline rats. These data implicate that repeated ketamine administration can induce behavioural alterations that are indicative of a hyperdopaminergic state.

Disclosures: A. Schumacher: None. **E.C. Tolledo:** None. **L. Ferguson:** None. **R. Ito:** None.

Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

Location: Halls B-H

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Topic: C.16. Schizophrenia and Bi-polar Disorder

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Loterie Romande

Title: Glutathione deficit affects white matter integrity in prefrontal cortex and impairs brain connectivity in schizophrenia

Authors: *A. MONIN[1], P. BAUMANN[2], A. GRIFFA[3], L. XIN[4], R. MEKLE[5], M. FOURNIER[1], C. BUTTICAZ[1], M. KLAEY[1], J.-H. CABUNGCAL[1], P. STEULLET[1], C. FERRARI[1], M. CUENOD[1], R. GRUETTER[4], J.-P. THIRAN[3], P. HAGMANN[3], P. CONUS[2], K. DO[1];
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Abstract: Schizophrenia pathophysiology involves neural dysconnectivity between the prefrontal cortex and posterior areas, likely related to oligodendrocyte and myelin impairments. Moreover, redox dysregulation induced by a glutathione synthesis deficit has been reported in the disease. As oligodendrocytes are highly vulnerable to oxidative stress, we investigated the interplay between glutathione levels and myelin in prefrontal cortex. In control subjects, multimodal magnetic resonance imaging revealed a positive correlation between glutathione levels in the anterior cingulate cortex and both white matter integrity (Pearson correlation r=0.52,

p=0.01) and functional connectivity of the cingulum bundle (Pearson correlation r=0.47, p=0.02). This correlation was disrupted in early psychosis patients. To substantiate the relationship between glutathione and myelin, we investigated myelin-associated proteins in mice with genetically impaired glutathione synthesis (*GCLM*-KO mice). Immunoreactivity of myelin markers was decreased by -44% in their anterior cingulate cortex at peripubertal age. At molecular level, a glutathione deficit induced by shRNA toward glutamate-cysteine-ligase catalytic subunit (GCL: key glutathione synthesis enzyme) reduced oligodendrocyte progenitor cell (OPC) proliferation by -26% and increase OPC early differentiation. The latter was mediated by an increase of Fyn kinase activity. Consistently, fibroblasts of patients having impaired glutathione synthesis presented an abnormal regulation of Fyn expression in response to oxidative stress. All together, these data indicate a critical role of glutathione and redox dysregulation in myelination processes and white matter tract maturation in the prefrontal cortex of rodent and human, a mechanism disrupted in schizophrenia.

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Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.04/HH6

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: JSPS

Title: A single minocycline administration suppresses methamphetamine-induced behavioral sensitization in mice

Authors: *T. A. KATO[1,2], S. KANBA[1], M. YAMATO[2,3], T. SHIBA[3], M. TAKARA[3], Y. SEKI[1], M. OHGIDANI[1], N. SAGATA[1], Y. YAMAUCHI[1], Y. MIZOGUCHI[4], K.-I. YAMADA[3], A. MONJI[4];
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Abstract: Introduction
Methamphetamine (METH), a functional dopamine agonist, is one of the most common abused

drugs, and METH abusers show various psychiatric symptoms especially psychosis. Repeated administration of METH produces a phenotype with enhanced locomotor activity, and these rodents show a behavioral sensitization to the locomotor enhanced by METH, which has been used as a model of stimulant psychosis. Minocycline, a tetracycline antibiotic, is known to have powerful anti-inflammatory and neuroprotective properties in animal models of various neurological disorders. In addition, minocycline has been highlighted as a potent psychotropic drug for schizophrenia.
Purpose/Methods
There are limited pharmacological treatments for METH-induced psychiatric symptoms. Thus, we herein examined the ability of minocycline to alter METH-induced behavioral sensitization. Behavioral sensitization is characterized by an enhanced locomotor response to METH after chronic administration has ceased. In this study, mice (adult male C57BL/6) received 5 mg/kg METH intraperitoneally (i.p.) for seven days. On Day 14, mice were challenged with 0.5 mg/kg METH to test for sensitization. Mice were placed in the center of an open field, and the number of line crossings was recorded as an index of locomotor activity.
Results
Mice were assigned randomly to three groups, each of which was designated as Control, METH and METH+ Single Mino. A seven-day repeated administration of METH significantly enhanced locomotor activity induced by METH challenge (0.5 mg/kg) on Day 14, signifying that METH sensitization had established. Surprisingly, a single-shot administration of 40 mg/kg minocycline i.p. immediately before METH challenge significantly inhibited locomotor activity.
Conclusion
This is the first report to show the inhibitory effect of single-shot minocycline on established methamphetamine (METH)-induced behavioral sensitization. Previous reports have shown that minocycline rescues METH-induced sensitization only when treated during the initial METH treatment period. Our novel finding further indicates that minocycline may be beneficial for the treatment of established METH-induced psychotic symptoms. Further investigations should be conducted based on our novel preliminary findings.

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Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

Location: Halls B-H

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Program#/Poster#: 255.05/HH7

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: MRC

Title: Modelling the cognitive function of the confirmed psychosis risk gene ZNF804

Authors: *J. E. HADDON, J. EDDY, T. AL-JANABI, M. O'DONOVAN, M. OWEN, L. WILKINSON;
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Abstract: ZNF804A was found to be a top hit for association with schizophrenia (SZ) in a recent Genome-Wide Association Study1 with the strength of association increasing when bipolar cases were added to the affected sample. The association between ZNF804A and SZ has since been replicated. Murine Zfp804a is orthologous with the human gene and thus lends itself to mouse models. Using ENU mutagenesis we have generated two mutations in Zfp804a, which we are studying on a number of behavioural tasks designed to reflect symptoms of SZ. Previous work has shown effects of the mutations on aspects of emotional and motivational function, including evidence consistent with anhedonia. We are now examining the effects of the mutations on murine analogues of the Stroop paradigm, a task requiring the use of contextual information to resolve response conflict, which SZ patients have difficulty with.
In Experiment One, C57BL/6 mice were trained concurrently on two discriminations, auditory and visual, in two discriminable contexts A and B, (e.g. A: Tone→Left lever, Clicker→Right; B: Flashing light→Left, Steady light→Right, counterbalanced between animals). Correct responses were rewarded with pellets in one context and sucrose in the other. Following acquisition, mice received test presentations of audiovisual compounds of the training stimuli in both contexts. The elements of these compounds required either the same (e.g. Tone and Flashing→Left) or different lever press responses (e.g. Tone and Steady→Left/Right) during initial training, termed congruent and incongruent stimulus pairs respectively. Mice demonstrated correct responding to the congruent stimulus pairs. Responding to the incongruent stimulus pairs was dependent upon the relationship between the elements of the audiovisual compounds and the test context, such that rats responded preferentially to the stimuli previously trained in the test context. For example, when tested in context A they responded based on the auditory cues (e.g. Tone and Steady→Left, Clicker and Flashing→Right) but in context B their responding was in accordance with the visual stimuli (e.g. Tone and Steady→Right, Clicker and Flashing→Left).
Previous evidence suggests that damage to the prefrontal cortex impairs the contextual control of response conflict, a region that might be influenced by these mutations. Experiment Two examined whether mutations to ZFP804a influence the ability to respond appropriately to response conflict in a manner similar to the cognitive impairments observed in patients with Schizophrenia performing the Stroop task.

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Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: EU and the European Regional Development fund (Polish Science Foundation grant MPD/2009/4)

Title: Abnormal high frequency oscillations in the nucleus accumbens in the MAM developmental rat model of schizophrenia

Authors: *S. ANTHARVEDI GODA, S. KASICKI, M. J. HUNT;
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Abstract: We have shown previously that spontaneous high frequency oscillations (130-180 Hz, HFO) were recordable from the nucleus accumbens (NAc) of awake rats and that NMDAR antagonists increase their power and frequency. Here, we examined whether HFO recorded in local field potentials (LFPs) of the NAc were altered in the MAM-E17 developmental rat model of schizophrenia. We found that the power and frequency of spontaneous HFO were significantly higher in MAM rats versus sham controls. In contrast the power of delta (<4 Hz) and broadband gamma (30-100 Hz) were not different between the groups. Injection of MK801 (0.05, 0.15, 0.3 mg/kg) dose dependently increased the power of spontaneous HFO in both groups. When we controlled for the larger HFO power at baseline, MK801 produced comparable magnitude of change in power for both group of rats. In contrast, MK801 did not affect the frequency of HFO in MAM rats but dose-dependently increased the frequency of HFO in the sham group. Additionally, we also found that MK801 increased the power of high gamma (70-90 Hz) in MAM rats alone. Taken together, these results suggest that abnormal spontaneous HFO are a characteristic feature of the MAM developmental model of schizophrenia.

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Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

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Program#/Poster#: 255.07/HH9

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: R01MH085635

Title: Neuroreceptor mechanisms of antipsychotic sensitization and tolerance

Authors: *S. CHOU, M. LI;
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Abstract: Chronic antipsychotic treatments often induce long-term alterations in their behavioral efficacy, termed antipsychotic sensitization and tolerance. Our previous studies indicated that repeated administration of olanzapine causes a sensitization effect in the conditioned avoidance response model, while clozapine causes a tolerance effect. However, molecular mechanisms underlying these lasting behavioral alterations are currently unclear. In the present study, we investigated the involvement of dopamine D2 receptors in antipsychotic sensitization and tolerance in a heterogenous group of animals. Male adult Sprague-Dawley rats born from dams treated with either saline or polyinosinic:polycytidilic acid during pregnancy to induce immune activation were first repeatedly treated with clozapine or olanzapine and tested daily for avoidance response for five consecutive days. Two days later the expression of antipsychotic sensitization and tolerance was examined in a challenge test in which all rats were injected with a lower dose of either drug. Finally, amphetamine was used to assess locomotor activity in all rats. We expected rats to display olanzapine sensitization and clozapine tolerance during both the repeated drug test days as well as the challenge test. We also expected rats previously treated with antipsychotics during the repeated drug test days to display significantly higher motor activity levels during the amphetamine-induced hyperlocomotion test compared to those treated with vehicle during the same period. We suggest that repeated antipsychotic treatment may stimulate dopamine D2/3 receptor up-regulation that induces lasting modulations of behavioral sensitivity to these drugs. Thus the results of this study shed light on possible receptor mechanisms involved in the long-lasting sensitization and tolerance effects caused by chronic antipsychotic treatment.

Disclosures: S. Chou: None. **M. Li:** None.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIMH Grant RO1MH91130

Title: Endocannabinoid-mediated modulation of neuronal correlates of social interaction in saline- and PCP-treated rats

Authors: *J. MATRICON, A. SEILLIER, A. GIUFFRIDA;
Pharmacol., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: The negative symptoms of schizophrenia (SCZ), which include anhedonia and social withdrawal, are resistant to pharmacological therapy. Experimental evidence suggests a link between negative symptoms and dysfunctional endocannabinoid transmission in the brain. Studies carried out in drug naïve schizophrenics have shown that the levels of the endocannabinoid anandamide (AEA) are inversely correlated to the severity of negative symptoms, suggesting that increased AEA production may be beneficial in SCZ. In line with this hypothesis, we previously showed that the social withdrawal observed in the sub-chronic phencyclidine (PCP) rat model of SCZ results from deficient stimulation of the cannabinoid CB1 receptor, and that AEA elevation induced by the fatty acid amide hydrolase inhibitor URB597 reverses this deficit via a CB1-mediated mechanism.

To identify the brain areas lacking CB1 stimulation during social interaction, we carried out an extensive analysis of c-Fos expression as a marker of neuronal activity in 28 cortical, limbic and subcortical regions relevant to SCZ and social behavior. Brain samples were collected from rats treated with PCP (5mg/kg, i.p., 2 injections/day for 7 days followed by 1-week wash out) or saline tested for social interaction in the presence (or absence) of URB597 (0.3mg/kg, i.p.). Rats exploring the social interaction arena alone were used as controls.

PCP treatment produced a social withdrawal, which was reversed by URB597. The same drug, however, produced social deficit in saline-treated rats. The behavioral phenotypes observed in these experimental groups matched the pattern of c-Fos activation in 2 out of the 28 cerebral regions studied. Specifically, social interaction produced neuronal activation in the orbitofrontal cortex of saline-treated rats, but not in PCP-treated animals. URB597 administration prevented the activation of this brain area in saline-treated rats, whereas it restored neuronal activation in the PCP-treated group. We found a reciprocal activation pattern in the central amygdala, suggesting a critical contribution of these 2 areas to the behavioral changes observed in our model.

To determine whether the c-Fos activation pattern reflects possible alterations of CB1 function, we are currently assessing changes in CB1 expression, activity and signaling in the orbitofrontal cortex and the amygdala using biomolecular techniques and subcellular fractionation.

These results will provide valuable information on the brain regions engaged during social

interaction in normal and schizophrenia-like conditions, and on the CB1 contribution to the neuronal activity of the underlying circuits.

Disclosures: J. Matricon: A. Employment/Salary (full or part-time):; UTHSCSA. **A. Seillier:** A. Employment/Salary (full or part-time):; UTHSCSA. **A. Giuffrida:** A. Employment/Salary (full or part-time):; UTHSCSA. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This research is supported by NIMH grant RO1MH91130 (AG).

Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.09/HH11

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: PHS grant MH-083729

Title: Working memory deficits in adult rats after acute elevation of brain kynurenic acid are alleviated by co-administration of the α7 nicotinic positive modulator galantamine

Authors: *S. A. VUNCK[1], K. SUPE[1], R. SCHWARCZ[2], J. P. BRUNO[1];
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Abstract: Schizophrenia (SZ) is a debilitating psychiatric disorder that affects ~1% of the world's population. Core cognitive deficits in thought processing, attention and working memory (WM) remain largely unresolved by current drug and behavioral interventions. While traditional pharmacotherapy has focused on dopamine antagonism, interactions among several cortical-subcortical neurotransmitter systems are dysregulated in SZ and offer potential targets for adjunctive drug therapy. Elevated kynurenic acid (KYNA) levels in the brain of patients with SZ may contribute to cognitive impairments through KYNA's negative allosteric modulation of α7 nicotinic acetylcholine receptors (α7nAChRs). This hypothesis has prompted the use of experimental elevations in brain KYNA to study the cognitive impairments seen in SZ. The present study used the operant delayed non-match to position (DNMTP) task in adult, male rats to determine the impact of acutely elevated KYNA on WM) and the ability of galantamine, a positive allosteric modulator (PAM) of the α7nAChR, to alleviate performance deficits. The DNMTP task has three behavioral components: sample, retention, and choice phases. The daily sessions consisted of 120 trials; delays were 5, 10 and 15 sec. Rats received 50 min pretreatment injections (i.p.) of 0, 25 or 100 mg/kg kynurenine (the bioprecursor of KYNA), with 48 hour

intervals to assure complete return of brain KYNA to baseline levels between doses (order randomized). Reduction of the deficits produced by 100 mg/kg kynurenine was attempted by systemic co-administration of galantamine (3 mg/kg, i.p.). Elevation of KYNA via acute administration of kynurenine resulted in significant main effects of dose and delay, with an interaction between the two factors. Compared to vehicle treatment, 25 mg/kg kynurenine significantly reduced accuracy (by 12%)) only at the longest delay (15 sec), while 100 mg/kg significantly reduced accuracy overall (17%) as well as at 5 sec (11%) and 10 sec (14%), with the 15 sec delay (33%) dropping to chance levels. The deficits following 100 mg/kg kynurenine were fully prevented by a 45 min pretreatment with galantamine. Our results show that the systemic administration of kynurenine results in significantly reduced, delay-dependent performance in the DNMTP WM task. This deficit appears to result from KYNA's negative allosteric modulation of α7nAChRs, as performance accuracy was normalized by galantamine. Further research using more selective α7nAChR agonists and PAMs will clarify the mechanisms of KYNA-related cognitive deficits in WM and may provide support for the continued focus on the α7nAChR as a target for cognition enhancement in SZ.

Disclosures: S.A. Vunck: None. **K. Supe:** None. **R. Schwarcz:** None. **J.P. Bruno:** None.

Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: PHS Grant MH-083729

Title: Chronic prenatal kynurenine elevation in rats: A naturalistic model of schizophrenia with biochemical abnormalities and deficits in hippocampal-mediated learning and memory

Authors: *A. POCIVAVSEK[1], M. A. R. THOMAS[1], G. I. ELMER[1], J. P. BRUNO[2], R. SCHWARCZ[1];
[1]Maryland Psychiatric Res. Center, Dept of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Departments of Psychology and Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Schizophrenia (SZ), a catastrophic psychiatric disorder, results from a combination of genetic and environmental factors. Kynurenic acid (KYNA), an endogenous antagonist of α7 nicotinic acetylcholine (α7nACh) and NMDA receptors, has been implicated in the pathology of SZ (elevated brain and CSF KYNA levels in SZ; genetic links to KYNA metabolism; role of

both α7nACh and NMDA receptors in neurodevelopment and cognition; etc.). The prominent neurodevelopmental hypothesis of SZ etiology is often studied in animals where the developmental period has been manipulated experimentally. In the present study, we increased KYNA levels prenatally, from gestational day (GD) 15 to GD 22, by adding the KYNA precursor kynurenine (kyn)(100 mg/day) daily to the chow fed to pregnant mothers (control: ECon; kyn-treated: EKyn). On the last day of treatment, KYNA levels in the brain of embryos were significantly elevated (ECon: 1344 ± 233 fmoles/mg protein, EKyn: 7654 ± 656 fmoles/mg protein, n=7 litters/group). Upon termination of the treatment, all rats were fed normal rodent chow until the animals were evaluated in adulthood, i.e. on postnatal days (PD) 56-80. KYNA levels were significantly elevated in the brain of EKyn offspring (ECon: 47 ± 4 fmoles/mg protein, EKyn: 104 ± 24 fmoles/mg protein, n=4-6 litters/group), and this was paralleled by a significant reduction in the activity of kynurenine 3- monooxygenase (KMO), a pivotal kyn pathway enzyme that controls the production of KYNA (ECon: 38 ± 8 pmoles/h/mg protein, EKyn: 6 ± 5 pmoles/h/mg protein, n=4-6 litters/group). To evaluate possible differences in de novo KYNA production, adult animals were acutely challenged with kyn (50 mg/kg, i.p., 90 min). This led to a significantly higher KYNA content in the brain of EKyn animals (ECon: 474 ± 22 fmoles/mg protein, EKyn: 702 ± 77 fmoles/mg protein, n=5-7 litters/group), possibly as a consequence of the low cerebral KMO activity. In separate animals, we tested hippocampus-dependent behaviors [passive avoidance paradigm (PAP) and Morris water maze (MWM)]. Prenatal kyn treatment caused significant PAP deficits, evidenced as decreased avoidance latency during the retention trial (ECon: 157 ± 32 s; EKyn: 43 ± 10 s; n=7 litters/group), and increased escape latency to find the hidden platform across days in the MWM (n=7 litters/group). Additional studies revealed that these delayed behavioral impairments are not seen when brain KYNA levels are raised experimentally in late adolescence (PD 42-49). Collectively, our results suggest that increases in brain KYNA during a vulnerable period in early brain development may play a significant role in the pathophysiology of SZ.

Disclosures: A. Pocivavsek: None. **M.A.R. Thomas:** None. **G.I. Elmer:** None. **J.P. Bruno:** None. **R. Schwarcz:** None.

Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: PHS Grant MH-083729 to JPB and RS.

Title: Prenatal kynurenic acid elevation alters cortical development and prefrontal glutamate release, corresponding to cognitive inflexibility in adults

Authors: *M. L. PERSHING[1], D. BORTZ[1], A. POCIVAVSEK[2], P. J. FREDERICKS[1], B. LEUNER[1], C. V. JØRGENSEN[3], R. SCHWARCZ[2], J. P. BRUNO[1];
[1]Psychology, The Ohio State Univ., Columbus, OH; [2]Maryland Psychiatric Res. Center, Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Neurobio. Res. Unit, Copenhagen Univ. Hosp., Copenhagen, Denmark

Abstract: Schizophrenia (SZ) is a debilitating neuropsychiatric disorder arising from neurodevelopmental alterations in brain circuitry. These changes underlie core deficits in cognitive control that are predictive of functional outcome yet are ineffectively treated with current pharmacotherapies. We have studied the effects of chronic elevations of kynurenic acid (KYNA) as a developmental model of SZ. KYNA, an endogenous antagonist of α7 nicotinic acetylcholine (α7nAChR) and NMDA receptors, is elevated in the brain of SZ patients and may contribute to cognitive deficits. We have reported that administration of KYNA's bioprecursor (kynurenine) from gestational day (GD) 15 through weaning produces deficits in hippocampal- and prefrontal-mediated tasks when offspring are tested as adults (Eur J. Neurosci., 35, 1605-1612, 2012; Neuroscience, 238, 19-28, 2013). In the present experiments, we attempted to further define the sensitive period for elevations in KYNA by limiting the dam's exposure to kynurenine (100 mg/day in mash) to the last prenatal week (GD15-22) (EKyn rats). KYNA levels were determined on GD21 and postnatal day (PD) 56. Cognitive flexibility was assessed between PD56-80 using an attentional set-shifting task (ASST). Given the role for prefrontal glutamate in set-shifting, animals from each litter were also used to determine the expression of mGluR2 receptor mRNA, the density of dendritic spines on cortical pyramidal neurons (layers II/III), and the mesolimbic control of prefrontal glutamate release using a glutamate-sensitive biosensor. EKyn rats had elevated brain KYNA levels, relative to controls (ECont), on GD21 (~5 fold) and PD56 (~1.5 fold). In adulthood, EKyn rats showed altered prefrontal glutamate transmission and exhibited deficits in the ASST at the first reversal and extra-dimensional shift stages. Relative to ECont, EKyn rats showed reduced expression of mRNA for mGluR2 receptor at GD21
(28%) and PD56 (27%) as well as reductions in apical (11%) and basal (14%) dendritic spine density of pyramidal neurons in layers II/III. Finally, the mesolimbic activation of prefrontal glutamate release following intra-accumbens infusion of NMDA seen in ECont rats (3.58 ± 0.40 µM increase at 0.15 µg NMDA) was nearly eliminated (0.43 ± 0.22 µM) in Ekyn rats. Thus, prenatal elevation of KYNA produces long lasting molecular and neuronal changes that are associated with disruptions in prefrontal development, cortical excitability, and cognitive flexibility. These results support a focus on elevated KYNA levels and altered cortical excitability as a mechanism underlying cognitive deficits in SZ.
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Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

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Program#/Poster#: 255.12/HH14

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: PHS Grant MH-083729

Title: Mesolimbic regulation of prefrontal glutamate release is blocked by local kynurenic acid and restored with oral administration of a kat ii inhibitor

Authors: *D. M. BORTZ[1], R. SCHWARCZ[2], J. P. BRUNO[1];
[1]The Ohio State Univ., Columbus, OH; [2]Maryland Psychiatric Res. Ctr., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Schizophrenia (SZ) is caused by a disruption between cortical and sub-cortical brain regions. Deficits in cognition, a core symptom cluster of the disease, are mediated by dysregulations in glutamatergic transmission in the prefrontal cortex (PFC). We have reported, using a glutamate biosensor (sampling frequency of 2 Hz; less than 1 sec to maximal response), that an infusion of NMDA into the rat nucleus accumbens (NAc) shell stimulates glutamate release in the PFC (Bortz et al, SFN 2012). Cognitively, this mesolimbic activation alleviates the performance deficits seen in rats during the distractor portion
of a sustained attention task (St. Peters et al, J. Neurosci., 225: 574-583, 2011). As glutamate levels in the PFC are reduced by increased levels of the neuromodulator kynurenic acid (KYNA; J.
Mol. Neurosci., 40: 204-210, 2010), and as brain KYNA levels are increased in individuals with SZ and result in cognitive deficits in animals, we tested the ability of KYNA to affect the stimulation of PFC glutamate by an intra-NAc NMDA infusion. As expected, local infusion of NMDA (0.15 µg) caused an elevation of glutamate in the PFC, with a mean amplitude of 4.27 ± 0.71 µM. Acute injections of
kynurenine (25, 50 or 100 mg/kg, i.p.), the bio-precursor of KYNA, 2 hr prior to the NMDA infusion dose-dependently suppressed the glutamate signal (mean % reduction of NMDA amplitudes: 71%, 86% and 94%; N=7, 3 and 3, respectively). As increased brain KYNA levels may play a causative role in the pathophysiology of SZ (Schiz. Bull., 36: 211-218, 2010), drugs designed to reduce brain KYNA formation represent a potential treatment approach. We

therefore targeted KAT II, the enzyme primarily responsible for the conversion of kynurenine to KYNA in the brain, in our paradigm. Oral administration of the
selective KAT II inhibitor BFF-816 (200 mg/kg), which readily crosses the blood-brain barrier (Wu et al., SFN 2012), delivered 2 hr prior to the NMDA infusion into the NAc, inhibited the suppressive effects of 50 mg/kg kynurenine (mean = 75 ± 11%) on the glutamate signal in the PFC by 37 ± 4% (N=5). These results provide further evidence that acute elevations in brain KYNA represent a good experimental model of SZ-like, behavioral deficits, and that KAT II inhibition may hold promise for future drug treatment of cognitive impairments in SZ.

Disclosures: D.M. Bortz: None. **R. Schwarcz:** None. **J.P. Bruno:** None.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.13/HH15

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: All authors are employees of AbbVie. The design, study conduct, and financial support for this work was provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Title: Two-hit model of schizophrenia: combining viral and glutamatergic insults

Authors: C. SCHIFANI, M. KLEE, B. JANSON, A.-L. RELO, *C. KLEIN, A. Y. BESPALOV;
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Abstract: Schizophrenia is a multifactorial disorder involving both genetic and environmental factors. A theoretical model was developed which describes the onset of the disease as an interplay of at least two “hits” during development. The present study focused on modeling this “two-hit hypothesis” to investigate potential compounding effects of maternal immune activation during pregnancy (polyinosinic-polycytidylic acid, Poly I:C, infusion on gestation day 15) and neonatal exposure of offspring to glutamatergic insult (repeated treatment with an NMDA receptor antagonist phencyclidine, PCP, on postnatal days 7, 9 and 11). Controls were treated with saline and left undisturbed before weaning. Several batches of male rats were used in the experiments and the most robust findings in the “two-hit” animals were increased locomotor activity when exposed to novel environment that persisted after habituation, reduced metabolic activity in dorsal and ventral hippocampus as well as in the prefrontal cortex (measured by [14C]2-deoxyglucose uptake), higher levels of pyruvate in the cerebrospinal fluid and enlarged

ventricles. In addition, various parameters of play behavior in juvenile rats were correlated to behavioral and neurochemical findings in the adulthood (e.g. animals that were less likely to engage in play behavior displayed higher activity in the open field). Furthermore, hippocampal metabolic activity was found to correlate with behavioral readouts in the 'two-hit' animals but not in controls (e.g. stronger glucose uptake in the hippocampus was associated with more activity in the open field). Taken together, combination of the perinatal poly I:C and PCP treatments results in robust and reproducible changes in behavioral, neurochemical and neuroanatomical parameters and therefore supports the two-hit hypothesis. Perhaps, most intriguing are the changes in the metabolic brain activity that correlate with behavioral measures that may be explored as a target for novel therapies of schizophrenia and related disorders.

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Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.14/HH16

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Comparison of Valproate acid and Haloperidol effects on excessive dopamine release in the amygdala in response to conditioned fear stress: Methamphetamine-sensitized rats

Authors: *H. MURAOKA, M. KAWANO, K. INADA, T. KAWANO, H. OSHIBUCHI, J. MIYAGI, A. KASAI, M. YAMADA, J. ISHIGOOKA;
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Abstract: Patients with schizophrenia may exhibit emotional hypersensitivity; therefore, it is meaningful to study dopamine dynamics in the amygdala. In our series of studies, we found that dopamine release in the amygdala was significantly increased in methamphetamine (MAP)-sensitized rats compared to control animals during a conditioned fear stress paradigm. Excessive dopamine release is considered a biochemical marker
of vulnerability to psychosis, and it is known that antipsychotic drugs antagonize dopamine signaling in the mesolimbic system. However, it is unclear how antipsychotic drugs affect

amygdalar function. In contrast,
mood-stabilizing drugs are used for augmentation therapy of schizophrenia and other emotional disorders, but there is no biochemical evidence that they exert different pharmacological effects on psychological stress. Therefore, we examined the differential effects of conditioned stress on basal dopamine release and response among control (saline; SAL), antipsychotic drug (haloperidol; HAL), and mood-stabilizer (valproic acid; VPA) groups.
Male Sprague Dawley rats received 2 mg/kg/day of MAP for 10 days to sensitize them to the drug, and a fear conditioning paradigm was conducted to model psychological stress. Dopamine changes in response to conditioned fear stress in the amygdala were measured by microdialysis and high-performance liquid chromatography.
Compared with SAL, both the HAL and VPA groups showed inhibition of excessive dopamine release in the amygdala. HAL elevated the number of dopamine vesicles in MAP rats, but VPA did not. These results suggest that VPA-mediated alteration in the number of dopamine-containing vesicles is one of the reasons why VPA is effective in ameliorating schizophrenia symptoms.

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Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NRSA Grant F31 DA034407-01

Title: The effects of phencyclidine on self-administration of nicotine: A novel hypothesis for the comorbidity of schizophrenia and nicotine abuse

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Abstract: Patients with schizophrenia smoke cigarettes at a disproportionately higher rate than the general population but the behavioral and neurobiological mechanisms underlying the comorbidity between schizophrenia and nicotine abuse remain unclear. The present experiment used preclinical means to test a novel hypothesis for this comorbidity: patients with schizophrenia are more sensitive to the reinforcement-enhancement effect of nicotine (i.e. the enhancement by nicotine of the reinforcing effects of other stimuli). This experiment

investigated this hypothesis by testing rats in an animal model of schizophrenia using a nicotine self-administration paradigm. Self-administration of nicotine is significantly increased when a cue light is presented concurrent with the nicotine infusion, which has been suggested as evidence for the reinforcement-enhancement effect of nicotine. Phencyclidine (PCP) was used to induce behavioral changes resembling symptoms of schizophrenia in rats and cue lights were either concurrently presented with the nicotine infusion during the self-administration session or absent from sessions. Forty male Sprague-Dawley rats were initially trained to lever-press for sucrose and then underwent catheter implantation surgery. They were then randomly assigned into four groups based on the drug injected prior to self-administration sessions and cue light availability: SAL-CUE, SAL-NO CUE, PCP-CUE, PCP-NO CUE. PCP (or the corresponding saline group) was administered every day before placement into the chamber. After responding stabilized, they underwent extinction and reinstatement with PCP and yohimbine, an alpha-2 adrenoreceptor antagonist, to determine what effect prior exposure to PCP would have on reinstatement of nicotine-seeking behavior. There were significant differences between groups on lever-pressing, suggesting that both cue exposure and previous administration of PCP contribute to changes in nicotine self-administration.

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Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

Location: Halls B-H

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Program#/Poster#: 255.16/HH18

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Early lifetime treatment with phencyclidine induces interneuron dysfunction and cognitive deficits in rats

Authors: *N. PLATH[1], B. V. BROBERG[2], C. KJÆRBY[1], A. S. KARLSEN[3], J. RIISE[3], P. H. LARSEN[1], J. P. REDROBE[1], L. LERDRUP[1];
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Abstract: Pharmacological inhibition of the NMDA receptor (NR) has been shown to induce psychosis-like symptoms in healthy controls and exacerbate psychotic symptoms in schizophrenia patients. Consequently, perturbation of rodents with NR antagonists, e.g. phencyclidine (PCP), has become a popular means to mimic schizophrenia-like symptomatology

in animals. One such approach, the neonatal (aka perinatal) PCP (neoPCP) model, additionally incorporates the neurodevelopmental dysfunction hypothesis of schizophrenia. We applied this model to address the effects of early life NR antagonism on cognition as well as a range of neuronal functions suggested to mediate cognitive processing.
To generate neoPCP rats, male Lister Hooded rats were treated with 20 mg/kg (s.c.) on postnatal days (PND) 7, 9 and 11 and underwent phenotypical assessment during early adulthood (PND 50 - 100).
As reported earlier, neoPCP treatment produced hypersensitivity to an acute NR antagonist challenge, as evidenced by increased locomotor activity and phMRI responses. Moreover, neoPCP rats displayed deficits in episodic memory (novel object recognition) and executive function (attentional set shifting) tasks, but not in classical conditioning and reversal learning tasks (touch screen visual discrimination). These cognitive deficits could partially be reversed with pro-cognitive agents shown to be efficacious on some cognitive domains in schizophrenia patients (e.g. Modafinil). On a cellular level, neoPCP treatment led to a 25% decrease of spine numbers in pyramidal neurons of the medial prefrontal cortex (mPFC), as well as an approximate 20% reduction in mRNA expression of the presynaptic marker SNAP25 in the hippocampal CA4 region. Furthermore, neoPCP animals presented an estimated 20% signal reduction for the interneuronal marker, parvalbumin. This result was mirrored by a significant decrease in miniature inhibitory postsynaptic potential (mIPSP) frequency in the mPFC layer II/III, but not layer V. Thus far, we did not see spontaneous recovery of any of these observations within the time frame studied.
In summary, the rodent neoPCP model is suggested to represent a valid perturbation to mimic some of the cognitive deficits related to schizophrenia, mediated at least in part by an impairment of interneuron functionality.

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Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.17/II1

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Title: Maternal immune activation causes age-specific increase in the number of microglia in offspring in rats

Authors: *K. SUZUKI, T. TAKAHASHI, K. YAMADA, Y. IWATA, N. MORI;
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Abstract: Background: Converging evidence from epidemiological, brain imaging, and neuropathological studies has suggested that at least in part of schizophrenia is a neurodevelopmental disorder, in which a brain abnormality is sustained early in life, but is not fully expressed until early adulthood. Among environmental factors that may destructively affect neurodevelopment, prenatal exposure to viral infection has been implicated by several large epidemiological studies, indicating that such exposure increases the risk of schizophrenia. However, it remains unknown the mechanism by which viral insults during prenatal period can cause latent development of schizophrenia. Based on post-mortem analysis showing an increased number of activated microglia in patients with schizophrenia, we hypothesized that these cells contribute to pathogenesis of the disorder.
Methods: Poly I:C injection of pregnant rats was used as an animal model of schizophrenia, and the number of microglia were assessed in the offspring at two developmental periods, prepubertal (four weeks after birth) and adulthood (eight weeks after birth), using anti-Iba1 immunohistochemistry.
Results: Eight- but not four-week-old offspring of pregnant dams exposed to 20 mg/kg of Poly I:C at embryonic day 12.5 showed significantly higher number of microglia in the hippocampus, frontal cortex, and striatum as compared to those of dams exposed to saline.
Conclusion: The result suggests that maternal immune activation during the prenatal period contributes to microglial activation after puberty in the offspring, and support the hypothesis that microglia is a contributing factor to the pathogenesis of schizophrenia.

Disclosures: K. Suzuki: None. **T. Takahashi:** None. **K. Yamada:** None. **Y. Iwata:** None. **N. Mori:** None.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.18/II2

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIMH R15MH098246

Title: Effects of decreasing cortical GABA transmission on decision-making in rats

Authors: *A. O'HARA[1], B. PLAUT[2], D. C. LOWES[2], T. A. PAINE[2];
[2]Neurosci., [1]Oberlin Col., Oberlin, OH

Abstract: Background: Schizophrenia is associated with a number of cognitive deficits including deficits in executive function and decision-making. These cognitive functions rely, in part, on the prefrontal cortex (PFC). Notably, pathology within cortical GABA interneurons that results in reduced GABA synthesis and release (i.e., reduced expression of the GABA synthesis enzyme GAD67 and increased expression of GABAA receptor subunits) is commonly observed in post-mortem analyses of the brains of people diagnosed with schizophrenia. Thus, the goal of this experiment was to determine if decreasing cortical GABA function leads to schizophrenia-like changes in decision-making in rats.
Methods: Male Sprague-Dawley rats were trained on a rodent gambling task (rGT) until they reached stable levels of individual performance and then were implanted with bilateral guide cannulae aimed at the medial prefrontal cortex (PFC). Following recovery, rats were trained until their individual performances restabilized and then tested following infusions of the GABAA receptor antagonist bicuculline (BMI; 0, 25, 50 ng/μl) or the GABA synthesis inhibitor L-allylglycine (LAG; 0, 10, 20 μg/μl).
Results: Blockade of GABAA receptors with BMI impaired decision-making. Following BMI (50 ng/μl) infusions, rats chose the most advantageous hole less frequently and made fewer total advantageous responses than vehicle (0 ng/μl) treated rats. In addition, BMI (50 ng/μl) infusions increased omissions relative to vehicle infusions. In contrast, inhibition of GABA synthesis with LAG (20 μg/μl) did not affect decision-making (there was no change in advantageous responding) but did increase impulsive behavior as indicated by an increase in premature responding.
Summary: Blockade of GABAA receptors, but not blockade of GABA synthesis, impaired decision-making as measured by the rGT. These data suggest that reduced GABA synthesis is not sufficient to explain the decision-making impairments observed in schizophrenia; instead they suggest that reductions in GABAA receptor activation may explain this cognitive deficit. Future research is aimed at determining why blocking post-synaptic GABAA receptors negatively impacts decision-making while decreasing GABA synthesis and release does not.

Disclosures: A. O'Hara: None. **B. Plaut:** None. **D.C. Lowes:** None. **T.A. Paine:** None.

Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.19/II3

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NIMH R15MH098246

Title: Chronic inhibition of cortical GABA synthesis does not impair attention

Authors: *T. A. PAINE;
Neurosci., Oberlin Col., Oberlin, OH

Abstract: Background: Attention deficits are a core feature of schizophrenia. Pathology of GABA interneurons within the prefrontal cortex (PFC), a brain region involved in optimal attention, is frequently observed in post-mortem analyses of brains from people with schizophrenia. It has been proposed that the net outcome of the pathological abnormalities is a reduction in GABA synthesis and release. The goal of the current experiment was to determine whether chronically reducing GABA synthesis within the rat medial PFC is sufficient to cause attention deficits as measured by the 5-choice serial reaction time task (5CSRTT). Because alterations in locomotor activity can have non-specific effects on 5CSRTT performance, we also examined the effects of chronically decreasing cortical GABA synthesis on locomotor activity in an open field.
Methods: Male Sprague Dawley rats were trained on the 5CSRTT until they reached criterion performance (>60% accuracy, <20% omissions for 5 consecutive days) and then were implanted with a bilateral cannula aimed at the medial PFC. Each cannula was connected to a osmotic mini pump that chronically infused the GABA synthesis inhibitor L-allylglycine (LAG, 3.2 μg/0.5 μl/hr) for 13 days. Following surgery rats were allowed to recover for 4 days and then were tested on the standard 5CSRTT for 5 consecutive days. Rats were then tested on a version of the 5CSRTT in which the stimulus duration was shortened (increasing attentional demands) and one in which the stimulus duration was lengthened (decreasing attentional demands). Finally, locomotor activity was measured assessed using an open field and then the rats were sacrificed. Brains were rapidly extracted and flash frozen for later analysis.
Results: Chronic LAG infusions did not affect accuracy of responding on any version of the task, but did increase omissions on both the standard and the short discriminative stimulus versions of the 5CSRTT. In addition, chronic LAG infusions increased premature responding on the standard version of the task. Finally, chronic LAG infusions slightly increased locomotor activity.
Summary: Chronic inhibition of GABA synthesis within the medial PFC, despite increasing impulsive behavior and locomotion, is not sufficient to impair attention per se. These data support our previous research in which acute inhibition of GABA synthesis within the medial PFC did not affect attention, yet increased locomotor activity. These data do not support the

hypothesis that disruptions in cortical GABA synthesis cause attentional impairments in schizophrenia.

Disclosures: T.A. Paine: None.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.20/II4

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Comparison of scopolamine and MK801 effects on touchscreen based cognitive tasks in rats

Authors: *T. M. BALLARD, R. WYLER, T. BURNS;
Neurosci. DTA, Pharma Res. & Early Development, F. Hoffmann-La Roche AG, Basel, Switzerland

Abstract: The touchscreen is a computer-automated behavioural test system which provides the opportunity to set up cognitive tests that are virtually identical to human and non-human primate tests and thus is highly relevant for translational research in schizophrenia. For the current study, separate cohorts of 12-16 male Lister Hooded rats were trained in Med Associates operant chambers equipped with touchscreens controlled by K-Limbic (Conclusive Solutions) to either: (1). Visual discrimination (VD) task using the IMI NEWMEDS protocol and conditions; (2). Paired associate learning (PAL) task (Talpos et al, 2009). In the VD task rats learnt to discriminate between two visual stimuli (spider vs. plane) and by 10-14 daily sessions had achieved a stable baseline level of >85% correct responses. PAL is a visual-spatial memory task in which rats were trained to associate spatial location (left, middle or right of screen) with the correct visual stimulus (spider, plane or flower), and required at least 60 daily sessions to reach a criterion of 85% correct responses. Once animals had achieved a stable baseline level of performance in each task, compounds were assessed using a full cross-over design. The basis of any touchscreen task requires successful discrimination of the visual stimuli and so the VD task was used to pre-screen compound effects and to select an appropriate dose-range for testing in the PAL task. In the VD task, scopolamine at 0.1 mg/kg s.c. significantly reduced percent correct responses and the number of trials completed during the session and increased the latency to make a correct response. MK801 at 0.1 mg/kg s.c. significantly reduced percent correct responses, total completed trials, and increased the latency to make a correct response, whilst 0.025-0.075 mg/kg significantly reduced the latency to collect the food pellet. In the PAL task,

scopolamine dose dependently reduced percent correct responses and number of trials completed at 0.05 and 0.075 mg/kg. Moreover, the same doses significantly increased the latency to make a response and to collect the food reward. In the PAL task, MK801 dose dependently reduced percent correct responses without affecting the number of trials completed at 0.025 mg/kg. At 0.075 mg/kg there was a significant increase in latency to make a response, whereas at 0.025-0.05 mg/kg there was a significant decrease in latency to collect the food reward. Scopolamine impaired memory at doses which did not have an impact on discrimination of the visual stimuli, but did impair performance of the task. However, MK801 at doses which did not affect performance of the task selectively impaired visuo-spatial memory in the PAL task.

Disclosures: T.M. Ballard: A. Employment/Salary (full or part-time):; F.Hoffmann-La Roche AG. **R. Wyler:** A. Employment/Salary (full or part-time):; F.Hoffmann-La Roche AG. **T. Burns:** A. Employment/Salary (full or part-time):; F.Hoffmann-La Roche AG.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Impairments in attentional performance and visual discrimination in the rat MAM model of schizophrenia

Authors: *D. YOUNG, W. M. HOWE, R. GRAF, S. M. O'NEILL, T. A. LANZ, R. KOZAK; Pfizer Inc, Cambridge, MA

Abstract: Schizophrenia is associated with a wide range of neurobiological and cognitive-functional abnormalities. However, identifying how disruptions across these domains specifically contribute to the etiology of the disease and may also serve as targets for treatment has been hindered by a lack of animal models that capture such broad symptomology. The gestational methylazoxymethanol (MAM) rodent model of schizophrenia is one that reproduces many of the structural, neurochemical, neurophysiological, and behavioral aberrations observed in schizophrenia (Lodge, 2013). We investigated the performance of MAM rats on two separate behavioral tasks that assess perceptual and attentional capacity: the visual discrimination task (VDT) and the sustained attention task (SAT). In the VDT, animals are presented with two stimuli and must press the correct image on a touchscreen in order to receive a food reward. MAM and sham controls were not significantly different in the time to acquire the task or on any performance measure. Once an animal reached criterion, the rules of the task were reversed, i.e.

the animal would need to press the opposite stimuli to receive reward; a total of two reversals were conducted. On trials that resulted in an incorrect response, MAM animals showed a significant increase in the latency to respond as compared to shams following a reversal. In the SAT, animals learn to press one of two levers which correspond to the presence or absence of a visual signal. MAM and sham controls displayed comparable levels of performance on the standard task. During the distracter version of the task (dSAT), in which a house light flashes on and off continuously at a rate of 0.5 Hz for a portion of the session, both groups were impaired similarly by the attentional challenge. However, MAM animals displayed a significant impairment in signal trial accuracy in the post-distracter recovery period of the session as compared to sham animals. These results suggest that the rat MAM model reproduces the impaired perceptual and attentional abilities observed in patients with schizophrenia. Studies are ongoing to assess the circuit bases of the behavioral disruptions observed here, with a particular focus on control of GABAergic and glutamatergic cortical circuitry by ascending dopaminergic and cholinergic systems. Together, the available evidence suggests that the MAM model can inform pre-clinical studies aimed at developing pharmacotherapies for the treatment of schizophrenia.

Disclosures: D. Young: A. Employment/Salary (full or part-time):; Pfizer Inc. Other; This work is part of the Innovative Medicines Initiative (IMI) project, Novel Methods leading to New Medications in Depression and Schizophrenia (NEWMEDS). **R. Kozak:** A. Employment/Salary (full or part-time):; Pfizer Inc. **W.M. Howe:** A. Employment/Salary (full or part-time):; Pfizer Inc. **R. Graf:** A. Employment/Salary (full or part-time):; Pfizer Inc. **T.A. Lanz:** A. Employment/Salary (full or part-time):; Pfizer Inc. **S.M. O'Neill:** A. Employment/Salary (full or part-time):; Pfizer Inc.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

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Title: Perineuronal nets protect fast-spiking interneurons against oxidative stress

Authors: *P. STEULLET[1], J.-H. CABUNGCAL[1], H. MORISHITA[2], R. KRAFTSIK[3], M. CUENOD[1], T. K. HENSCH[4], K. Q. DO[1];
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Abstract: A hallmark of schizophrenia pathophysiology is the dysfunction of cortical inhibitory GABAergic neurons expressing parvalbumin, a type of interneurons that are essential for coordinating neuronal synchrony during various sensory and cognitive tasks. High metabolic requirements of these fast-spiking cells may render them susceptible to redox dysregulation and oxidative stress. Using mice with a genetic redox dysregulation (*Gclm* KO mice with a reduced capacity of glutathione synthesis), we demonstrate that a specialized polyanionic matrix (perineuronal nets) enwrapping most of these interneurons as they mature, plays a critical role in the protection against oxidative stress. These nets limit the impact of genetically impaired anti-oxidant systems and/or excessive reactive oxygen species produced by severe environmental insults. A six-month redox dysregulation (as in 6 month-old *Gclm* KO mice) impairs many parvalbumin interneurons and their associated local neuronal synchrony, but spares those parvalbumin interneurons bearing well-formed perineuronal nets. We also observe an inverse relationship between the robustness of the perineuronal nets around parvalbumin interneurons and the degree of intracellular oxidative stress they display. Enzymatic degradation of the perineuronal nets renders mature parvalbumin interneurons (not calbindin and calretinin cells) and fast rhythmic neuronal synchrony more susceptible to oxidative stress. In parallel, parvalbumin interneurons enwrapped with mature perineuronal nets are better protected than immature parvalbumin interneurons surrounded by less condensed perineuronal nets. Thus, the high susceptibility of parvalbumin interneurons to oxidative stress during postnatal development is due in part to the yet incomplete maturation of this specialized extracellular matrix. While perineuronal nets act as protective shield, it is however itself sensitive to excess of oxidative stress. The protection might therefore reflect a balance between the oxidative burden on perineuronal net degradation and the capacity of the system to maintain the nets. Abnormal perineuronal nets as observed in *post mortem* brains of schizophrenia patients may thus underlie the vulnerability and functional impairment of pivotal inhibitory circuits in schizophrenia.

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Poster

255. Schizophrenia and Bipolar Disorder: Animal Models II

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Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: A double-dissociation between the effects of MK-801 and Scopolamine in two touch-screen based tasks of hippocampal dependent spatial memory

Authors: *J. C. TALPOS, III, J. OLLEY, T. STECKLER;
Neurosci., Janssen Pharmaceutica NV, Beerse, Belgium

Abstract: A cognitive test battery now exists for use in touch-screen equipped operant boxes. While this approach holds promise, an important consideration is the ability of these assays to detect distinct and dissociable profiles after discrete regional (e.g. lesion), transgenic, or pharmacological manipulations. Here we report the affects of glutamatergic and cholinergic receptor antagonists on performance of the trial unique non-match to location (TUNL) task, as well as a touch-screen based automated search task (AST).
Rats (male Lister-Hooded, Harlan) were trained in TUNL or AST performed in Med Associates operant boxes run by K-Limbic software (Conclusive Solutions). In TUNL, a sample location would be displayed on the screen and the rat was required to poke at it. After a delay of 1 or 20s the rat had to choose between the original location and a new location displayed on the screen (96 trials per session). Effects of delay and spatial separation between the stimuli on performance were considered. In AST, a rat had to acquire the position of a specific location on the screen to obtain reward. The rewarded location would remain the same for 10 trials and then shift, requiring the rat to again find the rewarded location (80 locations per session). Both tests have previously been shown to be sensitive to lesions of the hippocampus, but their underlying neuropharmacology is unexplored. Once steady state performance had been achieved in TUNL or AST, MK-801 (0.025-0.075 mg/kg) or scopolamine (0.025-0.05 mg/kg) was administered. MK-801 impaired percent correct at all doses in TUNL, with delay dependent effects at 0.025 mg/kg and delay-independent effects at higher doses. A significant separation dependent effect was not observed in TUNL, although a tendency for such an effect was seen at 0.025 mg/kg. Scopolamine impaired percent correct at all doses tested; however 0.05 mg/kg also decreased responding. In AST, MK-801 impaired performance across all doses tested, while 0.025 mg/kg interacted with trial number, causing no impairment on the initial trial, but impairing performance on subsequent trials. In contrast, scopolamine only impaired performance on the first trial of a block.

In summary, MK-801 and scopolamine were capable of inducing impairments in primary cognitive measures in TUNL as well as in AST, however the pattern of impairments differed between drugs and tasks. These data indicate that these tasks have unique neuropharmacological underpinnings, and likely measure different cognitive processes. When used together TUNL and AST provide an effective means to separate the overlapping processes of attention, working memory, and spatial pattern separation.

Disclosures: J.C. Talpos: A. Employment/Salary (full or part-time):; Janssen Research & Development. **J. Olley:** A. Employment/Salary (full or part-time):; Janssen Pharmaceutica NV. **T. Steckler:** A. Employment/Salary (full or part-time):; Janssen Pharmaceutica NV.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.24/II8

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: Innovative Medicines Initiative Joint Undertaking grant agreement n° 115008

Title: Mice carrying chromosomal microdeletions relevant to schizophrenia and performances on touchscreen tasks of learning and executive functioning

Authors: S. R. O. NILSSON, *L. M. SAKSIDA, T. W. ROBBINS, T. J. BUSSEY;
Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Chromosomal microdeletions at locations 22q11, 15q13 and 1q21 are among the largest known genetic risk factors for schizophrenia (Stone et al. 2008, Stefansson et al. 2008) and other psychopathologies. The current experiments tested transgenic (TG) mice carrying analogous microdeletions at chromosomal locations 22q11, 15q13 and 1q21 on touchscreen based tasks tapping learning and executive functioning. These tasks included the 5-choice serial reaction time task (5-CSRTT), used to assess attention and impulsivity, and two-choice visual discrimination and reversal learning, used to assess learning and cognitive flexibility.
15q13 TG mice showed attentional deficit in the 5-CSRTT with decreased accuracies at shorter stimulus duration. 1q21 TG mice showed impulsive responding in the 5-CSRTT with increased premature responses at longer delays. Although the genotype effect in the 1q21 TGs diminished with repeated testing, it could be restored with systemic amphetamine. 22q11 TG mice showed improved performance in visual discrimination, reversal learning, and the 5-CSRTT.
These data suggests that chromosomal microdeletions in mice affects cognitive functions relevant to schizophrenia.

Disclosures: S.R.O. Nilsson: None. **L.M. Saksida:** F. Consulting Fees (e.g., advisory boards); LMS consult for Camden Instruments. **T.W. Robbins:** 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.; Lilly, Lundbeck. F. Consulting Fees (e.g., advisory boards); Cambridge Cognition, Lilly, Lundbeck, Teva, Shire Pharmaceuticals, Merck Sharp and Dohme. Other; GSK Editorial: Springer Verlag. **T.J. Bussey:** F. Consulting Fees (e.g., advisory boards); TJB consult for Camden Intruments.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.25/II9

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: IMI academic-industry collaboration NEWMEDS

Title: Modafinil fails to improve neonatal PCP-induced cognitive deficits in rats

Authors: S. J. SUKOFF RIZZO[1], T. A. LANZ[1], G. LU[2], A. SHAO[2], X. YU[2], S. M. O'NEILL[1], *R. KOZAK[1];
[1]Neurosci. Res. Unit, Pfizer, Cambridge, MA; [2]WuxiAppTecWuxiApptec Inc, Shanghai, China

Abstract: Recent clinical and preclinical evidence has implicated a role for modafinil in the treatment of cognitive impairments associated with schizophrenia. We evaluated the ability of modafinil to reverse cognitive deficits induced by treatment of neonatal rats with the NMDA receptor antagonist phencyclidine (PCP) that have been shown to induce schizophrenia-like symptoms including cognitive impairment. The neonatal PCP model is a neurodevelopmental model of schizophrenia based on resulting biochemical, synaptic, and behavioral changes observed within subjects in adulthood (Wang et al 2001; Broberg et al 2008). Briefly, newborn Long-Evans rat pups were treated with PCP (20 mg/kg, s.c.; neoPCP) or saline vehicle (neoSHAM) on postnatal days 7, 9, and 11. On PND ~56, male neoPCP and neoSHAM treated controls were assessed in a habituated locomotor activity test after acute challenge with PCP (5mg/kg, s.c.). neoPCP subjects demonstrated a significant increase in locomotor activity after acute PCP administration relative to neoSHAM treated controls ($p<0.05$). neoPCP rats were identified as low and high responders to acute PCP challenge, as defined by those individual subjects whose data for total distance traveled were below and above the group mean, respectively. One week following acute PCP challenge, subjects in the high responder group

(n=25) were selected for evaluation of spatial learning and memory in the widely used water maze task. neoSHAM and neoPCP rats were randomized into treatment groups (10 or 32 mg/kg modafinil or vehicle control, p.o.). Daily pretreatment with modafinil (30 min prior to first acquisition trial) did not improve escape latency or distance traveled to reach the hidden platform during the acquisition phase of the task. As expected, neoPCP rats demonstrated impairments in spatial memory retention during the probe trial relative to the neoSHAM group. This deficit was not reversed by modafinil. Furthermore, a significant reduction in GABAergic transcripts (parvalbumin, GAD1, GAD2) in neoPCP rat were observed in frontal cortex compared to the neoSHAM controls. Taken together these data demonstrate that neonatal PCP treatment results in long term neuronal and behavioral changes including hypersensitivity to acute PCP challenge and cognitive deficits that are not reversed by modafinil treatment.

Disclosures: S.J. Sukoff Rizzo: None. **T.A. Lanz:** None. **S.M. O'Neill:** None. **R. Kozak:** None. **G. Lu:** None. **A. Shao:** None. **X. Yu:** None.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.26/II10

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NEWMEDS - The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115008 of which resources are composed of EFPIA in-kind contribution and financial contribution

from the European Union's Seventh Framework Programme (FP7/2007-2013).

Title: Dissecting the perceptual and cognitive profile of subchronic phencyclidine (PCP) administration in the rat using novel touchscreen-based assays

Authors: *A. C. MAR[1], S. BARBER[2], R. WOOLLEY[2], L. M. SAKSIDA[2], T. J. BUSSEY[2], T. W. ROBBINS[2];
[1]Behavioural and Clin. Neurosci. Inst., [2]Psychology, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Schizophrenia patients show disruption in a variety of domains including sensory perception, attentional processing and executive function. Administration of N-methyl-D-aspartate (NMDA) receptor antagonists in rodents has been proposed as an animal model of cognitive dysfunction in this disorder. We examined the effects of sub-chronic phencyclidine

(sPCP) treatment (5mg/kg, twice daily for 7 consecutive days) in touchscreen-based behavioural assays for rodents - including a novel continuous performance test (rCPT) in which subjects are required to continuously attend, identify and touch briefly-presented target stimuli but to withhold responding to non-target stimuli. Twenty-eight Lister hooded rats were allocated to two experimental conditions: one group was pretrained and matched for baseline rCPT performance prior to sPCP or vehicle treatment, and the other trained and evaluated on rCPT only following sPCP or vehicle treatment. After an eight-day minimum washout period, the groups were reassessed or trained under baseline conditions, respectively, and further examined on a series of probe tests involving alteration of the perceptual and/or cognitive difficulty. Across both experimental conditions sPCP-treated rats showed comparable rCPT performance to vehicle-treated controls on measures of attention and inhibitory control. However, sPCP-treated rats exhibited significantly decreased target hit rate and response criterion when stimulus contrast levels were reduced (e.g., 12.5-25%). This highly selective pattern of results suggests that sPCP might provide a model of perceptual deficits in the visual magnocellular pathway that have been proposed as a putative endophenotype in schizophrenia. The discovery of such perceptual abnormalities may better frame some of the diverse behavioral effects observed in the sPCP model and aid in the identification of novel neural substrates for therapeutic intervention. These findings highlight the flexibility and sensitivity of the novel rCPT paradigm and related measures as well as the utility of the touchscreen method for translational research in psychiatry.

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Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.27/II11

Topic: C.16. Schizophrenia and Bi-polar Disorder

Title: Down-regulation of mGluR2 and mGluR6 receptors in the frontal cortex of neonatal phencyclidine(PCP)-treated adult rats, a developmental model of schizophrenia, reflects decreased promotor methylation at histon H3K4me3

Authors: M. J. MILLAN, B. CHANRION, *E. SCHENKER, B. P. LOCKHART, C. MANNOURY-LA COUR;
Inst. De Recherches Servier, Croissy Sur Seine, France

Abstract: Epigenetic mechanisms are strongly implicated in neurodevelopmental disorders like autism and schizophrenia (1), but the precise changes and functional consequences remain poorly defined. A recent study demonstrated large-scale changes in neuronal trimethylated histone H3-lysine 4 (H3K4me3) remodelling occur in early childhood (2) highlighting the importance of the H3K4me3 mark in mechanisms of neurodevelopment. We used the neonatal phencyclidine (PCP) neurodevelopmental model of schizophrenia to analyze whether epigenetic changes during development may contribute to altered gene expression characteristic of schizophrenia. Rats were injected during neonatal days 7, 9 and 11 with 10 mg/kg s.c. PCP. The mRNA expression of 50 target genes in PCP-treated adult rats (8 weeks) was studied and significant (ca. 30%) decreases in mGluR2 and mGluR6 mRNA expression seen in frontal cortex (FCX) and hippocampus. In parallel, levels of H3K4me3, a chromatin mark associated with transcriptional activation, was significantly decreased at mGluR2 and mGluR6 promoters in these structures in PCP-treated *versus* control adult rats. PCP treatment also was found to decrease mGluR2 protein levels in the FCX. Interestingly, we also found that the H3K4me3 mark progressively increased at mGluR2 and mGluR6 promoters in the FCX of control rats from a juvenile age (8 weeks) adulthood, while no increase was seen in PCP-treated rats. Finally, we checked the level of H3K4me3 in neuronal and non-neuronal nuclei of the PFC, and the reductions in H3K4me3 seen in PCP-treated adult rats at the mGluR2 promoter were restricted to neurones, with H3K4me3 unchanged in non-neuronal nuclei. These findings suggest that epigenetic mechanisms, specifically a loss of H3K4me3 recruitment, contribute to reduced neuronal mGluR2 and mGluR6 mRNA expression in a neurodevelopmental rat model of schizophrenia.
1. Millan, MJ. An epigenetic framework for neurodevelopmental disorders, from pathogenesis to potential therapy. Neuropharmacology 68 (2013) 2-82.
2. Akbarian, S, et al., Epigenetic regulation in human brain - focus on histone lysine methylation. Biol Psychiatry 65, 198-203.This work was partially performed in conjunction with the European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initiative under Grant Agreement n°115008

Disclosures: **M.J. Millan:** A. Employment/Salary (full or part-time):; IdR Servier. **B. Chanrion:** A. Employment/Salary (full or part-time):; IdR Servier. **E. Schenker:** A. Employment/Salary (full or part-time):; IdR Servier. **B.P. Lockhart:** A. Employment/Salary (full or part-time):; IdR Servier. **C. Mannoury-La Cour:** A. Employment/Salary (full or part-time):; IdR Servier.

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.28/II12

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: Innovative Medicines Initiative Joint Undertaking 115008

Title: Tackling behavioural variability: A case study with the MAM E17 rodent model of schizophrenia

Authors: *N. N. MALIK, F. GASTAMBIDE, M.-C. COTEL, B. EASTWOOD, S. FITZJOHN, J. LI, F. PASQUI, K. PHILLIPS, J. SHERWOOD, M. J. O'NEILL, G. GILMOUR, M. TRICKLEBANK;
Lilly Ctr. For Cognitive Neuroscience, Eli Lilly & Co Ltd, Surrey, United Kingdom

Abstract: One of the major challenges in schizophrenia research has been the development of suitable models for the cognitive aspects of the disorder. In this regard, one model that has been studied in some detail is the methylazoxymethanol (MAM) neurodevelopmental model. Our findings and those of other laboratories confirm that the MAM E17 model offspring exhibit several neurodevelopmental and pathological changes that bear similarities to schizophrenia. These include reductions in cortical thickness and hippocampal size, and enlargement of ventricles. Electrophysiological properties and sleep physiology are also affected, shown by changes in ES-coupling, sleep bout length and delta power. Behavioural consequences of MAM administration, largely emergent after puberty, include increased locomotor responsiveness to psychostimulant administration, pre-pulse inhibition and cognitive flexibility deficits.
However, the robust neuropathological and neurophysiological findings found in the MAM E17 model do not always translate into behavioural deficits and we have found them to be highly variable. This is of critical consequence for model validation and use in discovery research. In the present study we decided to tackle some of these issues by assessing key neuroanatomical (hippocampal volume, cortical thickness and ventricular enlargement), neurophysiological (electrophysiology, sleep and EEG) and behavioural outcomes (prepulse inhibition, reversal learning and fear conditioning) in a large pool of 240 rats coming from 66 different litters. This design allowed us to perform a correlation analysis to assess if deficit in one assay could predict a deficit in another assay and also to assess within litter correlations. The variability in behaviour effects will be discussed as well as study design, litter effects and statistical analysis.
Overall, our data suggest that maternal treatment with MAM on embryonic day 17 leads to persistent alterations in the adult offspring of Sprague Dawley rats that are relevant for modelling aspects of schizophrenia - but revised methods for study design and statistical analysis are crucial to avoid misinterpretation of findings.

Disclosures: N.N. Malik: A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **F. Gastambide:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **M. Cotel:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **B. Eastwood:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **S. Fitzjohn:** A.

Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **J. Li:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **F. Pasqui:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **K. Phillips:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **J. Sherwood:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **M.J. O'Neill:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **G. Gilmour:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd. **M. Tricklebank:** A. Employment/Salary (full or part-time):; Eli Lilly & Company Ltd..

Poster

255. **Schizophrenia and Bipolar Disorder: Animal Models II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 255.29/II13

Topic: C.16. Schizophrenia and Bi-polar Disorder

Support: NEWMEDS

Title: Attentional deficits following postnatal NR1 knock-out in corticolimbic interneurons

Authors: M. HVOSLEF-EIDE[1], L. LYON[2], A. C. MAR[2], K. NAKAZAWA[3], L. M. SAKSIDA[2], *T. J. BUSSEY[1];
[1]Dept. of Exptl. Psychology, [2]Deparment of Exptl. Psychology, Univ. of Cambridge, Cambridge, United Kingdom; [3]Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Mouse models that focus on simulating the cognitive deficits observed in patients with schizophrenia are increasingly becoming available. Thorough understanding of the nature of the deficits displayed is essential both for validation of the models as well as for pre-clinical drug screening. Recently it has been shown that postnatal (but not adult) knockout (KO) of the NR1 subunit of the NMDA receptor in 40-50%
of corticolimbic interneurons of mice is sufficient to produce post-adolescent schizophrenia-related phenotypes (Belforte et al., 2009). The extent to which these mice display further cognitive impairments, and the degree to which such impairments relate to those observed in patients, is currently unknown. Using touchscreen-equipped operant chambers, the ability of NR1 KO mice to acquire a visual discrimination and reversal was investigated. Additionally, attentional capacity was probed using the 5-Choice Serial Reaction Time Task (5CSRTT) and a novel rodent version of the continuous performance task (CPT). Compared to littermate controls, NR1 KO mice were significantly impaired on the CPT. This impairment in CPT performance could not be explained by simple discrimination impairments, as standard visual discrimination and reversal did not differ from controls. Furthermore, only a mild deficit could be detected in

the 5CSRTT, suggesting a specific deficit when required to attend to a single location involving the detection of a visual signal against noise. The specificity of the impairment in a task highly relevant to schizophrenia suggests that the manipulation of corticolimbic interneuron functionality at a critical developmental stage could be a valuable model of schizophrenia-like cognitive dysfunction.

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Jiang, Z., Yu, G., Li, Y., Quinlan, E.M., Nakazawa, K., 2009. Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nature Neuroscience, 13* (1), 76-83.

Disclosures: M. Hvoslef-Eide: None. **L. Lyon:** None. **A.C. Mar:** None. **K. Nakazawa:** None. **L.M. Saksida:** None. **T.J. Bussey:** F. Consulting Fees (e.g., advisory boards); Campden Instruments.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.01/II14

Topic: C.18. Drugs of Abuse and Addiction

Support: R37-006214

P50 DA 016511

Title: Sex differences in the role of brain corticotropin releasing factor (CRF) and norepinephrine (NE) in drug seeking during initial abstinence

Authors: *A. M. CASON, A. BOURREZA, M. W. FELTENSTEIN, G. ASTON-JONES; Med. Univ. South Carolina, CHARLESTON, SC

Abstract: Brain locus coeruleus NE (LC-NE) and CRF neurons are involved in stress responses, including the ability of stress to drive drug relapse. Previous animal studies indicate that female rats exhibit greater drug seeking than male rats during initial drug abstinence. Moreover, females are more sensitive to the effect of stress to drive drug seeking than males. Finally, brain LC-NE neurons are more sensitive to the stress peptide CRF in females compared to males (Curtis et al., 2006). We hypothesized that this increased drug seeking in females on extinction day one (ED1) is due to an increased response to the stress of early withdrawal (initiation of abstinence) and is dependent upon the increased response of LC in females to CRF. Therefore, we tested the

hypothesis that pretreatment with CP 154,526, a CRF receptor antagonist, would decrease drug seeking on ED1 as measured by responding on an active lever previously associated with cocaine self-administration. Male and female rats were trained to lever-press for iv cocaine in an operant chamber in 2-h daily sessions. After 10 self-administration sessions in which they earned ≥ 10 infusions, rats underwent a 90-120 min test for extinction responding by measuring lever pressing in the absence of cocaine reward (ED1). Twenty min prior to this extinction session, rats were injected (ip) with vehicle or CP 154,526 (CP), to measure effects of CRF antagonism on drug seeking during early abstinence. Pretreatment with the CRF1 receptor antagonist CP (10-20 mg/kg) decreased cocaine seeking on ED1 to similar levels in male and female rats. These findings indicate that signaling at CRF receptors are involved in the increased drug seeking during initial abstinence.
Following the ED1 cocaine seeking test, rats were killed and their brains were processed with immunohistochemistry to identify NE and CRF neurons that were Fos-activated during the ED1 cocaine seeking test. Our preliminary findings indicate that there is increased Fos expression in LC-NE neurons in vehicle treated female rats (n=4) compared to male rats (n=3) rats on ED1. Ongoing studies are examining the effects of pretreatment with CP on Fos expression in LC and NE neurons in other brain areas of male and female rats.

Disclosures: A.M. Cason: None. **A. Bourreza:** None. **M.W. Feltenstein:** None. **G. Aston-Jones:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.02/II15

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA016511

Title: Role of oxytocin neurons in mediating the behavioral response to early extinction from cocaine self-administration: Comparisons between female and male rats

Authors: *M. E. SMITH, A. M. CASON, G. ASTON-JONES;
Dept Neurosci, MUSC, CHARLESTON, SC

Abstract: Several studies find that drug seeking on the first day of extinction (Extinction Day 1, ED1), after a period of cocaine self-administration, is substantially greater in female compared to male rats. Moreover, stress responses, and drug relapse propensity, is greater in women than in men. The first day of extinction is important as it represents the initiation of abstinence, which is

stressful due to absence of chronic drug. The hypothalamic neuropeptide oxytocin (OT) is involved in reward and "anti-stress" processes; for example, OT release is stimulated by stress (Plotsky 1987). Therefore, it is possible that OT cells are affected by the stress of initial abstinence, and may contribute to male-female differences in early drug seeking.
Male, Sprage-Dawley rats were implanted with indwelling jugular catheters and trained to self-administer cocaine iv (daily 2 hr sessions, 0.2mg/kg/ infusion). Following 10 days of stable FR1 responding (>25 infusions/session) rats were placed in the self-administration chamber for an initial extinction session (2 hr, no cocaine infusions given) or remained in their home cages. Rats were then deeply anesthetized, transcardially perfused with saline and 4% paraformaldehyde, and the brains were removed and processed for immunohistochemical localization of Fos and OT.
Preliminary results indicate that the expression of Fos in OT neurons is decreased in male rats subjected to ED1 training compared to similar animals that instead remained in their home cages. Such a decrease in the activity of OT neurons may contribute to the negative effects of the period of initial abstinence; experiments with females are underway to evaluate these results with males because of known sexual dimorphisms. Given the role of norepinephrine (NE) and corticotropin releasing factor (CRF) systems in stress responses, and interactions of these systems with OT neurons, we will also examine Fos in NE and CRF neurons on ED1 and test whether NE or CRF neurons play a role in the response of OT neurons on ED1.
We hypothesize that treatments that modulate OT transmission during the early withdrawal from cocaine abuse may facilitate initiation, as well as subsequent maintenance, of abstinence, particularly in female addicts.

Disclosures: M.E. Smith: None. **A.M. Cason:** None. **G. Aston-Jones:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.03/II16

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant R37-DA006214

NIH Grant F32-DA026692

NIH Grant K99-DA035251

Title: Dopamine DREADDs: Chemicogenetic control of midbrain dopamine activity during reinstatement of cocaine seeking

Authors: *S. V. MAHLER, E. M. VAZEY, G. ASTON-JONES;
Neurosciences, Med. Univ. of South Carolina, CHARLESTON, SC

Abstract: Ventral Tegmental Area (VTA) is crucial for many reward-related behaviors, and both dopamine and non-dopamine neurons there play complex roles in reward and motivation. Although VTA dopamine neurons are clearly involved in reward, a lack of specific tools to selectively manipulate dopamine neurons has limited our ability to test their specific functions in behaving animals.
Designer receptors exclusively activated by designer drugs (DREADDs) are synthetic G-protein coupled receptors that are inert, except in the presence of their agonist, CNO (which is otherwise pharmacologically inert). DREADD-expressing neurons can therefore be experimentally controlled in a highly selective, "lock-and-key" manner. DREADDs can be targeted to VTA or substantia nigra dopamine neurons via local microinjections of viral vectors containing a floxed DREADD gene into transgenic rats, whose dopamine neurons express Cre recombinase (TH::Cre rats). This approach allows "remote control" of dopamine neuron activity via systemic injections of CNO.
Here, we use viral vectors to express DREADDs in VTA dopamine neurons, and in their inputs from ventral pallidum, in TH::Cre transgenic rats. We expressed excitatory or inhibitory DREADDs (Gs, Gq, and Gi-coupled) in midbrain dopamine cell populations, and examined effects on cue-induced or cocaine-primed reinstatement of cocaine seeking, and on VTA cell firing patterns. We also examined effects of manipulating the reinstatement-related inputs from ventral pallidum to VTA dopamine neurons on reinstatement and VTA cell firing. Results reveal that VP is a critical input to VTA for cued reinstatement of cocaine seeking.
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Disclosures: S.V. Mahler: None. **E.M. Vazey:** A. Employment/Salary (full or part-time):; Medical University of South Carolina. **G. Aston-Jones:** A. Employment/Salary (full or part-time):; Medical University of South Carolina.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

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Program#/Poster#: 256.04/II17

Topic: C.18. Drugs of Abuse and Addiction

Support: PHS grant R37-DA006214

Title: Specific roles of hippocampal and lateral septal neurons in context- vs. cue-induced reinstatement of cocaine seeking: Fos and DREADD studies

Authors: *E. M. MCGLINCHEY, G. ASTON-JONES;
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Drug relapse often occurs when addicts are re-exposed to drug-associated contexts or discrete drug cues. In a rodent model of addiction, this is modeled by context- or cue-induced reinstatement of extinguished drug seeking. The dorsal hippocampus has been found to be necessary for context-, but not cue-induced reinstatement, suggesting different circuits may mediate these two modalities. Recent evidence from our lab revealed a functional circuit between the hippocampal CA3 neurons to the ventral tegmental area (VTA), via a relay in the caudal-dorsal lateral septum (cdLS). As the CA3 region is important for contextual processing and the VTA in reward processing, this circuit indicates the cdLS may be important for linking contextual information with reward seeking. It is clear from early self-stimulation studies in both humans and rodents that the lateral septum plays a role in reward-driven motivation; however, this brain region has been largely understudied in drug abuse and relapse. This study aims to understand the role of the hippocampus-lateral septum circuit in the context- vs. cue-induced reinstatement of cocaine seeking. A modified self-administration model of addiction was designed to dissociate between the drug-associated contextual environment and the discrete light/tone cues paired with cocaine infusions during training. All rats trained to self-administer cocaine with light/tone cues in one context, extinguished this behavior in an alternative context (without light/tone cues), and either reinstated in the training context without the light/tone cues (context reinstatement, ABA) or in the extinction context using the light/tone cues (cued reinstatement, ABB). Fos expression was used as a marker of neuronal activation in the hippocampus (CA3, CA1, and dentate gyrus) and lateral septum (cdLS, caudal LS, rostral LS) following context or cued reinstatement tests. Fos expression was greater in all lateral septum and dorsal hippocampal sub-regions for context- compared to cue-induced reinstatement. Furthermore, preliminary data indicate that inhibition of LS neurons using Designer Receptors Exclusively Activated by Designer Drug (DREADD) technology may attenuate context-induced, but not cue-induced reinstatement. Together these findings indicate that the hippocampal-LS circuit may be important for drug reward-environment associations that drive addicts to relapse.

Disclosures: E.M. McGlinchey: None. **G. Aston-Jones:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.05/II18

Topic: C.18. Drugs of Abuse and Addiction

Support: R01-DA022658

R37-DA006214

T32-DA007288

Title: Oxytocin reduces the motivation to self-administer methamphetamine in a novel within-session behavioral-economic paradigm: Male-female comparisons

Authors: *B. M. COX, B. S. BENTZLEY, C. M. REICHEL, R. E. SEE, G. ASTON-JONES; Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Human and animal studies indicate that females have greater motivation to use methamphetamine (meth), which may contribute to enhanced relapse vulnerability. Women initiate meth use at a younger age and transition faster to dependence than men. Similarly, female rats acquire meth self-administration at higher rates and exhibit greater meth intake than males. Under a progressive ratio schedule of reinforcement, female rats show more motivation to seek meth. However, factors that influence the desired levels of intake (e.g., tolerance, sensitization) must also be considered when measuring motivation for drug, and the progressive ratio test does not account for intake differences associated with such factors. Behavioral-economic (BE) models have been designed to assess changes in consumption as a function of effort and provide separate measures of meth intake (Q_0) and motivation normalized to intake (α, a measure of demand elasticity). These measures have been shown to predict relapse in both human and animal studies, indicating that BE models will further our understanding of the relationship between motivation to seek drug and relapse behavior, and facilitate screening novel pharmacotherapies for treatment of addiction. Thus, utilizing a within-session BE paradigm, we used these measures to directly compare males and female rats during meth self-administration. We also tested oxytocin, a potential antirelapse medication, for its ability to attenuate meth seeking in both sexes. Females showed greater motivation to seek meth (lower α) compared to males and higher meth intake (higher Q_0). Oxytocin decreased motivation to seek meth in both sexes, but did not alter their intake at null price. Correlations between behavioral-economic and reinstatement measures, and determination of whether oxytocin during the within-session BE paradigm can predict attenuation of subsequent reinstatement, will also be presented. Overall, this novel paradigm will help to delineate sex differences observed in motivation to seek meth and may predict the efficacy of pharmacotherapies to treat meth addiction.

Disclosures: B.M. Cox: None. **B.S. Bentzley:** None. **C.M. Reichel:** None. **R.E. See:** None. **G. Aston-Jones:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.06/JJ1

Topic: C.18. Drugs of Abuse and Addiction

Support: R01-MH092868

R37-DA006214

F30-DA035065

Title: Adaptive demand: DREADD mediated stimulation of locus coeruleus during a within-session behavioral economics procedure

Authors: *Z. A. COPE[1], B. S. BENTZLEY[1], E. VAZEY[1], B. L. ROTH[2], G. S. ASTON-JONES[1];
[1]Neurosci., Med. Univ. of South Carolina, Charleston, SC; [2]Pharmacol., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Modern theories of behavioral control posit a pivotal role for locus coeruleus-norepinephrine (LC-NE) neurons in appropriately directing attention according to utility and task demands. The Adaptive Gain Theory (AGT; Aston-Jones and Cohen, Ann Rev. Neurosci 2005) states that when task utility (reward value) is high it is adaptive to focus attention on task completion, and that such focused attention is facilitated by low tonic (baseline) LC activity with high phasic (bursting) responses associated with decision completion preceding a behavioral response. Conversely, when the utility of an ongoing behavior wanes, it becomes adaptive to disengage from the task to seek out other tasks with possibly greater reward. The AGT proposes that phasic firing of LC decreases and tonic activity increases to facilitate task disengagement in the face of sustained low utility. Behavioral-economic methods measure dynamic changes in demand as a function of price, i.e. the effort required of a rat to obtain a reward. Here, we employed a within-session behavioral-economic procedure to assess changes in demand for cocaine as a function cocaine price (lever responses/mg cocaine). At high prices, the utility of performing the behavior wanes, and motivation no longer supports the increased effort needed to obtain reward. The maximum price that maintains increases in response rate is known as Pmax. We hypothesize that high tonic discharge of LC-NE neurons causes attention to be disengaged from a task at a lower Pmax than lower tonic LC-NE activity. LC-NE neurons were tonically activated using the excitatory hM3Dq DREADD receptor (Designer Receptors Exclusively Activated by Designer Drugs). This DREADD was selectively expressed in LC-NE neurons with in vivo microinjections of a viral vector containing the synthetic dopamine beta hydroxylase

promoter, PRSx8. Morphological and electrophysiological studies showed this vector to be effective in transducing and activating LC-NE neurons (see Vazey and Aston-Jones, this meeting). After cocaine self-administration training, animals were randomized to receive one of three doses of the selective DREADD agonist, clozapine-N-oxide (CNO; 0, 1, or 10 mg/kg, ip) 30 min prior to testing in the within-session behavioral economic procedure. A decrease in Pmax in response to CNO would provide evidence for a causal role of LC in modifying behavior in response to changing task demands and utility. Further results and conclusions will be discussed. Supported by PHS grants R01-MH092868, R37-DA006214 and F30-DA035065.

Disclosures: Z.A. Cope: None. **B.S. Bentzley:** None. **E. Vazey:** None. **G.S. Aston-Jones:** None. **B.L. Roth:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.07/JJ2

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant R37-DA006214

NIH Grant T32-DA007288

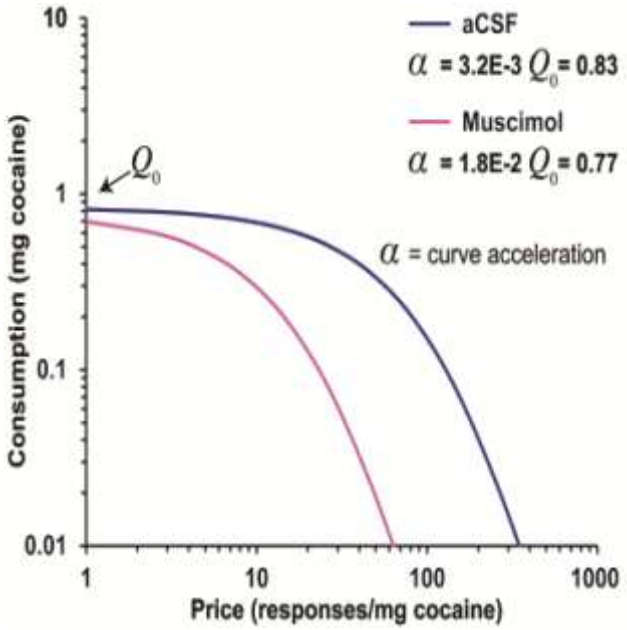
NIH Grant F30-DA035065

Title: Inactivation of the subthalamic nucleus decreases the economic essential value of cocaine

Authors: *B. BENTZLEY, G. ASTON-JONES;
Med. Univ. of SC, Charleston, SC

Abstract: Economic essential value of a drug has been shown to correlate with several clinical measures of addiction. In an animal model of relapse, essential value predicts cue-induced reinstatement of methamphetamine and cocaine seeking. The current study determined the role of the rat subthalamic nucleus (STN) in driving the economic essential value of cocaine. Consumption of cocaine was measured at 11 ascending cocaine prices (lever responses/mg cocaine) in a single 110-min session. Rats were pretreated with bilateral microinjections (0.3 μL) into STN of either vehicle (artificial cerebrospinal fluid) or the $GABA_A$ receptor agonist muscimol (0.2 mM) immediately prior to sessions in a within-subjects crossover design. Muscimol pretreatment significantly attenuated the essential value of cocaine compared to vehicle or injections of muscimol immediately dorsal to STN. In contrast, muscimol pretreatment

did not reduce cocaine consumption when the price of cocaine remained low throughout the session (e.g., FR1 schedule), indicating that STN inactivation results in price-dependent changes in cocaine consumption. Further, muscimol treatment did not alter locomotor activity in a novel environment. Given the clinical promise of economic measures of drug use, these results support a possible clinical utility of STN inactivation in treating cocaine abuse - akin to STN deep brain stimulation for treatment of Parkinson's disease, obsessive-compulsive disorder and major depression. Supported by NIH grants R37-DA006214, T32-DA007288, and F30-DA035065.



Disclosures: B. Bentzley: None. **G. Aston-Jones:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.08/JJ3

Topic: C.18. Drugs of Abuse and Addiction

Support: R37 DA006214

T32 DA07288-22

Title: Conditioning with highly palatable food rewards: A role of orexin on attention?

Authors: *L. R. FREEMAN, G. ASTON-JONES;
Neurosciences, Med. Univ. of South Carolina, CHARLESTON, SC

Abstract: Orexin neurons provide a well-known link between regulating vital functions and body energy status, but they are also known for their major input to arousal and reward centers. In fact, lateral hypothalamic orexin neurons have widespread connections throughout the brain. In the current study, orexin neurons and the effects of their projections to the medial prefrontal cortex (mPFC) were evaluated for conditioned responding to highly palatable food rewards. Male Sprague-Dawley rats were divided into two groups; one group was trained to lever-press for standard chow pellets ("Chow") and the other group was trained to lever-press for a high-fat, sucrose pellet ("HFS") on a fixed-ratio 1 schedule (FR-1). Subsequently, all rats were switched to a Go/No-Go paradigm with a new, highly palatable reward: chocolate-flavored sucrose. Preliminary results reveal no change in Go trial success in either group, but increased success on No-Go trials for HFS-conditioned rats. These results reveal a potential improvement in attention for food-motivated tasks after HFS training. In a recent study by Figlewicz et al. (2013), moderate high fat diet consumption increased sucrose self-administration compared to chow-fed control rats. The Sclafani group reported that lateral hypothalamus (in the area of orexin neurons) is involved in flavor-nutrient conditioning (2002), and another study has shown that infusions of orexin into mPFC can improve attention of rats (Furudono et al. 2006). From these and other results, we hypothesize that rats previously trained with HFS exhibit greater attention and/or motivation during subsequent Go/No-Go training due to elevated orexin transmission. We are currently testing this hypothesis by examining Fos induction in orexin and mPFC neurons. Future studies will further test the role of orexin utilizing an orexin antagonist with this paradigm. Supported by PHS grants R37-DA006214 and T32 DA07288-22.

Disclosures: L.R. Freeman: None. **G. Aston-Jones:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.09/JJ4

Topic: C.18. Drugs of Abuse and Addiction

Title: Palatable food entrainment: A model of food addiction in rats

Authors: *S. GONZALEZ-GARCIA[1], S. BLANCAS[2], G. ESTRADA[2], M. HERNANDEZ[2], L. UBALDO[2], C. ESCOBAR[2];
[1]UNAM Facultad de Medicina, Ciudad De Mexico, Mexico; [2]Anatomia, Facultad de Medicina, Mexico City, Mexico

Abstract: Recent reports suggest that consumption of palatable food induces similar neuroadaptive responses as drugs in the reward system. ΔFosB is a stable, long lasting protein believed to be involved in these changes. When 5g. of chocolate are offered daily at the same hour for up to 3 weeks rats exhibit anticipatory activity expecting chocolate access and this behavior persists for many days after the protocol is interrupted. The aim of this study was to determine if this behavior can reflect a process of addiction. Therefore we assessed whether rats would exhibit effort to obtain chocolate and determined changes of ΔFosB expression in the reward system. We also determined anticipatory changes in general activity and body temperature. Methods. Male Wistar rats (250 g) were housed in individual cages in a 12/12 cycle, fed with chow and water ad libitum. Intraperitoneal iButtons programmed to collect temperature were implanted. Rats assigned to chocolate entrainment received for 21 days 5g. of chocolate at 13:00. Brains were obtained of rats after chocolate ingestion or during chocolate anticipation and were compared with control undisturbed rats. Brains were prepared for immunohistochemistry for ΔFosB. We designed a wire box cube in which we put the 5 g piece of chocolate. This allowed the animals to see and smell the chocolate but could not touch, scratch or bite it. The aim was to determine the effort of rats to obtain the snack. After 3 weeks of chocolate entrainment rats exhibited anticipatory activity in behavior and body temperature and exhibited increased expression of ΔFosB in the nucleus accumbens, basolateral amygdala and prefrontal cortex. They also exhibited significant effort events to obtain chocolate. Conclusion: Scheduled access to chocolate entrains the circadian system and produces changes at the neuronal and behavioral level similar to those observed in drug abuse.
This study was supported by CONACyT 82462 and DGAPA-PAPIIT IN-224911.

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Poster

256. **"Learning, Memory, Dependence, and Addiction"**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.10/JJ5

Topic: C.18. Drugs of Abuse and Addiction

Support: NSERC

Title: Operant intraoral self-administration of high fructose corn syrup in laboratory rats

Authors: *A. LEVY[1,2], C. LIMEBEER[2], J. FERDINAND[2], U. SHILLINGFORD[2], L. PARKER[2], F. LERI[2];
[1]Psychology, [2]Univ. of Guelph, Guelph, ON, Canada

Abstract: The similarities between obesity and drug dependence suggest the hypothesis that "addiction" can develop to food. The current studies investigated whether principles of behavioral pharmacology that regulate intake of drugs of abuse also apply to intake of sweets. Rats implanted with intraoral cannulas were allowed to self-administer sweet solutions directly in their oral cavity. High fructose corn syrup (HFCS) was selected because of its association with overeating and obesity.
First, it was determined that intraoral self-administration (IOSA) was concentration-dependent. That is, intake was higher when the concentration of HFCS was lower (25% vs 50%). At an intermediate concentration (8%), responding was more variable, and tests using the progressive ratio schedule indicated a positive linear relationship between concentration and break points achieved. Second, self-administration of HFCS was regulated by the nutritional consequences of IO infusions. In fact, animals adjusted intake when the concentrations were altered. Third, taste/palatability also played a role in IOSA. In fact, higher concentrations engendered higher hedonic reactions measured by taste reactivity tests. This role, however, was significantly modulated by the caloric value of the reinforcer because when rats self-administered a concentration of saccharin that produced hedonic reactions similar to those induced by 25% HFCS, self-administration was very low. In addition, rats trained on 25% HFCS stopped responding when 0.1% w/v saccharin was substituted for HFCS.

The sensitivity of IOSA to differences in concentrations and schedules of reinforcement makes it an ideal procedure to investigate features of addictive-like behaviors engendered by voluntary intake of sweets.

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Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

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Topic: C.18. Drugs of Abuse and Addiction

Support: Klarman Family Foundation Grants Program in Eating Disorders Research

Title: Prefrontal cortical neuroadaptations following self-administration and reinstatement of highly-palatable food: The role of brain-derived neurotrophic factor

Authors: *S. M. BARRY[1], M. N. HUIZENGA[2], G. SADRI-VAKILI[2], J. F. MCGINTY[1]; [1]Neurosci. Inst., Med. Univ. of South Carolina, Charleston, SC; [2]MassGeneral Inst. for Neurodegenerative Dis., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Obesity has become an increasingly prevalent public health problem in the United States. Recently there has been a shift in understanding obesity and studies are now focused on the psychological factors involved in the development of the disease. Brain-derived neurotrophic factor (BDNF) has an integral role in glucose regulation, satiety, and control of food consumption. There is extensive evidence that altered BDNF signaling plays a role in chronic overconsumption of food to the point of obesity. Selective BDNF knockdowns have further implicated BDNF in selectively enhanced palatable food intake. We used an operant model of animal food self-administration to examine the differences in food self-administration and reinstatement between groups of Sprague Dawley rats lever pressing for either standard chow pellets or palatable chocolate pellets with an enhanced sucrose content. Our results indicate that BDNF protein levels in the dmPFC, an area highly implicated in control of reward seeking behavior, was decreased immediately following 7 weeks of self-administration of palatable chocolate pellets. Additionally, preliminary evidence indicates a possible down regulation in total mRNA of BDNF's activity dependent exon IV transcript in dmPFC. In a separate cohort, we found that rats will escalate their lever pressing to receive palatable pellets but not standard chow pellets over the last 5 weeks of self-administration. Following extinction of lever pressing, both groups of rats reinstated with a cue and pellet prime. However rats that self-administered standard chow pellets reinstated to a greater degree than those that self-administered the chocolate pellets. Furthermore, pellet and cue primed reinstatement of palatable food seeking produced greater activation of the prelimbic (PrL) cortex than reinstatement of standard chow seeking. These data provide both a role for BDNF and the PrL in palatable food self-administration and seeking, respectively.

Disclosures: S.M. Barry: None. **M.N. Huizenga:** None. **G. Sadri-Vakili:** None. **J.F. McGinty:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

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Topic: C.18. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program

NH&MRC Early Career Fellowship 1053308

Title: Context-induced reinstatement to alcohol seeking after punishment is associated with activation of nucleus accumbens shell projections to lateral hypothalamus

Authors: *N. J. MARCHANT, R. RABEI, J. M. BOSSERT, A. BONCI, Y. SHAHAM;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Background: Alcoholics typically abstain because of negative consequences associated with excessive drinking. During abstinence, relapse is often triggered by exposure to contexts previously associated with alcohol use. To model this condition, we developed an animal model of context-induced relapse where alcohol seeking is suppressed by adverse consequences (punishment; Marchant et al., 2013). Here, we identify activated projections from nucleus accumbens (NAc) shell to lateral hypothalamus (LH) during relapse after punishment.
Methods: We injected the retrograde tracer cholera toxin subunit B (CTb) into LH of alcohol-preferring (P) rats to label NAc shell neurons projecting to LH. Next, we trained rats to lever-press for 20% alcohol in context A, followed by mild-shock punishment of alcohol self-administration in context B. After punishment, we tested rats under extinction conditions without shock in either context A (ABA) or context B (ABB); a third control group (ABO) was not tested. After testing, we anesthetized, perfused, and extracted brains of rats to measure Fos (neuronal activity marker) and CTb+Fos in NAc shell.
Results: Alcohol seeking (lever-presses) was higher in group ABA than group ABB, demonstrating context-induced relapse after punishment. This relapse was associated with higher Fos expression in LH neurons, as well as a higher percentage of LH projecting NAc shell neurons (Fos-Ctb double-labeled neurons).
Summary: Context-induced relapse to alcohol seeking after punishment is associated with activation of both LH neurons and NAc shell neurons that project to LH. We are currently examining the causal role of NAc shell to LH projections in this relapse.

Disclosures: N.J. Marchant: None. **R. Rabei:** None. **J.M. Bossert:** None. **A. Bonci:** None. **Y. Shaham:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

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Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA029815

Title: Effects of amphetamine exposure during adolescence or adulthood on reward devaluation

Authors: *E. R. HANKOSKY[1], N. M. KOFSKY[1], C. HONG[1], J. M. GULLEY[2];
[1]Psychology, [2]Psychology & Neurosci. Program, Univ. Illinois Urbana-Champaign, Champaign, IL

Abstract: Drug use typically begins in adolescence, which is a period of ongoing neurobiological development that may confer heightened vulnerability to develop drug dependence. Previously, our lab has shown that amphetamine (AMPH) exposure during adolescence alters orbitofrontal cortex (OFC)-sensitive behaviors, such as behavioral flexibility and reversal learning. Here, we investigated the sex- and age of exposure-dependent effects of AMPH on an OFC-sensitive outcome devaluation task. Male and female Sprague-Dawley rats, born from breeders maintained in our facility, were injected (i.p.) with saline or 3 mg/kg AMPH every other day between postnatal day (P) 27-45 and P85-103. On P125, rats were trained twice daily for 10 days on a Pavlovian conditioning task where a conditioned stimulus (CS; tone or flashing lights) was paired with a specific reward (orange- or grape-flavored sucrose). Subsequently, one of the two rewards was devalued (via 2 hours of free access) and rats were then given a devaluation test session consisting of 5 presentations of each CS delivered under extinction conditions. Twenty minutes prior to the devaluation test, rats were injected with vehicle or 1 mg/kg SB 242084 (5-HT2C antagonist). Our preliminary data suggest that females and adolescents treated with AMPH continue to exhibit conditioned responses to the CS paired with a devalued outcome, suggesting their behavior is driven by reward-associated cues rather than the value representation of the reward. This may be due to alterations in 5-HT signaling as pretreatment with SB 242084 partially restored outcome devaluation in these groups. Overall, our preliminary results suggest that AMPH exposure impairs OFC-sensitive cognitive flexibility in females and rats exposed during adolescence via deficits in 5-HT2C receptor function.

Disclosures: E.R. Hankosky: None. **N.M. Kofsky:** None. **C. Hong:** None. **J.M. Gulley:** 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.; Abbott Laboratories.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.14/JJ9

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA029815

Title: Amphetamine exposure during adolescence alters synaptic plasticity in young adulthood via adaptations in dopaminergic signaling

Authors: *S. KANG[1,2], C. L. COX[2,3,4], J. M. GULLEY[2,5];
[2]Neurosci. Program, [3]Dept. of Mol. and Integrative Physiol., [4]Dept. of Pharmacol., [5]Dept. of Psychology, [1]Univ. of Illinois At Urbana Champaign, Champaign, IL

Abstract: Our previous studies suggest that repeated exposure to amphetamine (AMPH) during adolescence induces long-lasting changes in dopamine receptor function in the medial prefrontal cortex (mPFC). Given the key role of dopamine in regulating synaptic efficacy, we hypothesized that adolescent AMPH exposure would have enduring effects on neuronal plasticity in the mPFC. To test this, we treated male Sprague-Dawley rats with saline or 3 mg/kg AMPH (i.p.) every other day from postnatal day 27 to 45 (10 injections total). Three to four weeks later, rats were sacrificed and tissue slices containing the mPFC were prepared for field potential recordings. In saline-treated rats, we found high frequency stimulation (HFS) at 50 Hz induced long-term depression (LTD). Application of the D_1 antagonist SCH23390 (10 µM) blocked the induction of LTD. In these slices, the same tetanic stimulation produced long-term potentiation (LTP) in the presence of low- concentration $GABA_A$ receptor blockade (bicuculline methiodide, BMI; 0.1 µM). In AMPH pre-treated rats, HFS led to LTP without BMI, suggesting that adolescent AMPH exposure induces a long-lasting impairment of inhibitory modulation in the mPFC. To investigate the role of altered D_1 signaling in this effect of AMPH exposure, we performed whole-cell path recording of deep layer pyramidal cells in the mPFC. In slices from controls, we found a significant increase in the frequency of spontaneous inhibitory post-synaptic currents (sIPSCs) following activation of D1 receptors by SKF38393 (10 µM) application; this effect was not observed in slices from AMPH exposed rats. Taken together, our data suggest that adolescent AMPH exposure impairs D_1-mediated modulation of inhibitory transmission in the PFC, which in turn has a long-lasting disruptive effect on synaptic plasticity. This drug-induced predisposition to LTP may alter normal cognitive functioning as the PFC network would be expected to be relatively more “noisy” and unable to filter and process information appropriately.

Disclosures: S. Kang: None. **C.L. Cox:** None. **J.M. Gulley:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

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Topic: C.18. Drugs of Abuse and Addiction

Support: NIH R01-MH073689

NIH F32-DA030831

Title: Repeated oxycodone exposure impairs reversal behavior when contingencies change rapidly

Authors: *K. M. SEIP-CAMMACK, M. SHAPIRO;
Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Persistent drug seeking in drug-abstinent individuals may reflect a general problem with behavioral inflexibility, a difficultly disengaging from ongoing behavior. This inflexibility may be caused by inaccurate encoding of reward history and thereby disrupts organized, goal-directed behaviors. While exposure to illicit drugs is associated with inflexible responding, less is known about the effects of prescribed opiate analgesics, such as oxycodone, on flexible, adaptive responses. The present study investigates the degree to which prolonged exposure to oxycodone alters sensitivity to reward history when contingencies change frequently or infrequently. We hypothesized that oxycodone-exposed rats would be impaired at updating reward representations, regardless of how often contingencies changed, and predicted that this impairment would slow learning and increase perseverative responding for previously rewarded stimuli. Adult male rats were trained to enter a specific goal arm on a radial arm maze to find food. After reaching a criterion of eight consecutive correct trials, they were given 24 additional trials to establish stable performance. The next day, the rats were exposed repeatedly to oxycodone (3mg/kg, 2x/day) or vehicle for five days. On the first drug-free day (Day 6), stable performance on the initial task was confirmed. On Days 7-8, the goal arm was changed at different frequencies. During low-frequency reversals (LFRs), rats were trained to a criterion performance of eight consecutive correct trials, followed by an additional 12 trials, before the goal arm was changed. During high-frequency reversals (HFRs), the goal arm changed after the rat made three consecutive correct choices. Before drug exposure, all rats rapidly acquired the initial task; after drug exposure, all rats learned new stimulus-reward associations (reversals) during the LFR task and showed equivalent stable performance on each new goal arm. In contrast, during the HFR task, oxycodone-exposed rats required more trials to learn rapid reversals and made more perseverative errors than controls, especially on the first reversal. The effect of oxycodone exposure varied with training history, so that rats trained first in the HFR task were not impaired in either HFR or LFR tasks. Thus, oxycodone exposure may impair rats' ability to update response strategies when they have a history of stable reward contingencies. In rats given a relatively stable reward history, this impairment may contribute to persistent responding for previous rewards, including drugs of abuse. **Support:** F32-DA030831 to KSC and R01-MH073689 to MS

Disclosures: K.M. Seip-Cammack: None. **M. Shapiro:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

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Program#/Poster#: 256.16/JJ11

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant RO1DA015687

Title: L-type calcium channel blockade in the VTA disrupts cocaine-associated contextual memory

Authors: *M. DEGOULET[1], C. E. STELLY[1], K.-C. AHN[2], H. MORIKAWA[1];
[1]Waggoner Ctr. For Alcohol and Addiction Res., Austin, TX; [2]Dept. of Psychiatry, Brain Res. Ctr., University of British Columbia at Vancouver, BC, Canada

Abstract: Development of drug addiction involves a maladaptive form of learning and memory in which drug-related experiences are remembered powerfully, resulting in persistent and uncontrollable drug seeking behavior even when the drug becomes unavailable during abstinence or extinction. We have shown previously that long-term potentiation (LTP) of NMDA receptor-mediated glutamatergic transmission onto dopamine (DA) neurons in the ventral tegmental area (VTA) is an important substrate for such learning and memory processes. L-type Ca2+ channels (LTCCs) control synaptic plasticity in different brain regions. Indeed, systemic/intracerebroventricular administration of LTCC antagonists has been shown to suppress the development of conditioned place preference (CPP) induced by psychostimulants and other drugs of abuse. Although CaV1.2 LTCCs are the predominant subtype in the brain, recent studies indicate important roles of CaV1.3 LTCCs in DA neuron Ca2+ signaling and psychostimulant-induced neuroadaptations. Therefore, we investigated the effects of CaV1.2 and CaV1.3 LTCC antagonists, applied directly into the VTA, on different phases of cocaine-induced CPP (10 mg/kg, i.p., three conditioning sessions) in male Sprague Dawley rats. Intra-VTA injection of the CaV1.2/CaV1.3 LTCC antagonist isradipine (0.6 pmol/0.3 µl/side) before each cocaine conditioning session completely abolished the acquisition of CPP. To test the effect of isradipine on CPP expression, rats were first subjected to CPP conditioning and 1st post-conditioning test (i.e., CPP expression test), then intra-VTA isradipine injection was made before the 2nd posttest. Although isradipine had no effect on CPP expression, CPP was completely extinguished when 3rd and 4th posttests were performed on the next two days without isradipine injection. Furthermore, previously isradipine-treated animals displayed no cocaine-induced

reinstatement when cocaine was injected before the 5th posttest. Intra-VTA injection of the newly developed selective CaV1.3 LTCC antagonist "compound 8" (6 pmol/0.3 µl/side; provided by Dr. Jim Surmeier at Northwestern University) also facilitated CPP extinction and prevented cocaine-induced reinstatement. In VTA slices, we found that isradipine (2 µM) had no effect on NMDA EPSCs or NMDA-dependent burst firing in DA neurons, consistent with the ineffectiveness of isradipine on CPP expression. We hypothesize that LTCC antagonists might interfere with the acquisition and maintenance of drug-associated contextual memories by affecting induction and reversal processes of NMDA LTP in the VTA.

Disclosures: M. Degoulet: None. **C.E. Stelly:** None. **H. Morikawa:** None. **K. Ahn:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.17/JJ12

Topic: C.18. Drugs of Abuse and Addiction

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ANPCyT, PICT 2008-2019, Argentina

NIH grant GM103425

Title: Modafinil rescues methamphetamine-Induced cognitive deficits in object recognition memory in mice: Role of ERK1/2 phosphorylation in prefrontal cortex

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Abstract: Chronic use of methamphetamine (METH) leads to long-lasting cognitive dysfunction in humans and animal models. Modafinil is a wake-promoting compound approved for the treatment of sleeping disorders and is being prescribed off label for the treatment of METH dependence. In the present study, we evaluated deficits in visual memory and ERK1/2

phosphorylation in the prefrontal cortex of mice treated chronically with METH; we also tested the ability of modafinil to rescue METH-induced cognitive and biochemical impairments in mice. After METH treatment (1mg/kg, sc, once daily for 7 days), mice performed the Novel Object Recognition (NOR) task, which evaluates visual memory and is sensitive to METH treatment. After the last METH injection, mice performed the NOR task which consisted of 3 habituation sessions to the open field (5 min a day, 3 consecutive days), training session (10 min, day 4), and a retention session (5 min, day 5). One hour before the training session, mice were given a single acute dose of modafinil (90 mg/kg, ip) or vehicle, and were exposed to two identical objects. After a 24 hr delay, mice performed the retention session where one of the objects was replaced by a new object. Sessions (time spent with objects and locomotor activity) were analyzed using an automated video tracking system (Ethovision XT7, Noldus). A preference index was calculated as the time spent exploring the novel object/total exploration time. Control and modafinil mice showed elevated preference indices compared to METH-treated mice ($p<0.05$). Acute modafinil increased the preference index in the NOR in METH-treated mice to values comparable to control ($p<0.05$). We also measured ERK1/2 phosphorylation in medial prefrontal cortex of METH- and saline-treated mice that were exposed to novel objects in the training session, compared to mice placed in the open field without objects. The same modafinil/vehicle acute dose was given 1 hr before testing and mice were sacrificed immediately after the 10 min session. Elevated ERK1/2 phosphorylation was found in medial prefrontal cortex of control mice exposed to objects ($p<0.05$), which was absent in METH-treated mice. Acute modafinil treatment had no effect by itself, but was able to restore the ERK1/2 phosphorylation induced by novelty in METH-treated mice to values comparable to controls ($p<0.05$). We demonstrated that modafinil induced a compensatory effect against METH-induced cognitive impairments, possibly by normalizing ERK1/2 signaling pathways in prefrontal cortex. Our results are of translational value since modafinil may be a valuable pharmacological tool for the treatment of cognitive deficits observed in human METH abusers.

Disclosures: **B. Gonzalez:** A. Employment/Salary (full or part-time):; Postdoctoral Award, Fundacion Bunge y Born, Argentina.. **M. Raineri:** None. **N. Colettis:** None. **J. Cadet:** None. **E. Garcia-Rill:** None. **F.J. Urbano:** None. **V. Bisagno:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

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Topic: C.18. Drugs of Abuse and Addiction

Support: CNRS

University of Bordeaux

ANR

Région Aquitaine

Title: Rewarding drug-induced activation of the VTA elicits a long-lasting preferential use of procedural learning strategies

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Abstract: The multiple memory systems hypothesis posits that different neural circuits function in parallel and may compete for information processing and storage. For example, instrumental conditioning depends on the striatum, whereas spatial memory is processed by a circuit centered on the hippocampus. However, the nature of the task itself is not sufficient to select one system over the other. As compared to aversive events, the impact of rewards on interactions between memory systems remain poorly understood. We have implemented an experimental set-up to compare the long-term effects of food (crisps) and drug (intra-VTA morphine) rewards on various forms of memory. This paradigm is based on a Y-maze discrimination task which can be acquired using either a cued or a spatial learning strategy. Subsequent use of cued and spatial learning strategies were further assessed using a competitive version of the Morris water-maze task. Behavioral testing was completed with brain analysis of the phosphorylated form of cAMP Response Element Binding (pCREB) protein, and recording of field potentials evoked by stimulation of the amygdalo-striatal and amygdalo-hippocampal, CA1 / Dentate Gyrus (DG) pathways in freely-moving mice. Synaptic plasticity was measured before or 1, 15 and 60 min after completion of sessions 1, 5 and 10 of the Y-maze task. We found that drug-induced activation of the reward system impaired spatial but not cue-guided learning. This spatial impairment was related to a dramatic decrease in pCREB expression within the dorsal hippocampus (mainly CA1 subfield) and the prefrontal cortex. This learning profile was related to a learning- and reward-dependent long-term potentiation (LTP) of neuronal activity in the amygdalo-striatal pathway, and a long-term depression (LTD) within the amygdalo-hippocampal pathways (dorsal CA1 and DG). Both food and drug rewards activated CREB in the nucleus accumbens. In contrast, only drug reward persistently upregulated CREB expression within the dorsal striatum for 72h. At the same time point (72h after the last Y-maze session), we observed a preferential use of cued-learning strategy in the water-maze. Decreasing CREB activity by infusing Rp-cAMPS into the dorsal striatum re-established a balanced expression of cued and spatial learning strategies. We conclude that drug-induced activation of the VTA persistently

configures memory systems interactions to promote the control of behavior by conditioning processes. This drug-induced cognitive bias could play a critical role in the instatement of addictive behaviors.

Disclosures: M. Husson: None. **M. Baudonnat:** None. **J. Guillou:** None. **M. Corio:** None. **D. Béracochéa:** None. **R. Vouimba:** None. **V. David:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

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Program#/Poster#: 256.19/JJ14

Topic: C.18. Drugs of Abuse and Addiction

Support: PHS grant DA-033572

Title: Characterization of Arc expression in corticolimbic areas associated with cocaine-induced conditioned locomotor activity

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Abstract: Environmental cues associated with drugs of abuse cause relapse among addicts. Conditioned activity (CA) refers to a Pavlovian paradigm in which repeated, non-contingent pairings of an activating psychostimulant drug with a particular environment produce enhanced locomotion upon exposure to this environment in subsequent drug-free tests. Previously, we used the conditioned place preference (CPP) model to show that the retrieval of a cocaine-context associated memory results in enhanced activity-regulated cytoskeletal-associated (Arc) expression in distinct corticolimbic regions. While the CPP model is frequently used to examine the molecular mechanisms underlying cocaine-cue memories, few studies have examined cocaine CA memories. Here, we examine *Arc* induction during the retrieval of a cocaine-context association, which is *not* based on preference. Male Sprague-Dawley rats were subjected to a contextual conditioning protocol in which they received cocaine (12 mg/kg; i.p.) or saline (1 ml/kg) paired with either the activity chamber or the home cage. Animals in the cocaine-paired context group were given cocaine prior to placement in the activity chamber for thirty minutes and saline two hours later in their home cage, for seven trials in each environment. Animals in the cocaine-unpaired context group received saline prior to placement in the activity chamber and cocaine in their home cage. Two days after conditioning, both groups were exposed for six minutes to the activity chamber in a drug-free state. A second paired group, activity-yoked

paired, was used to control for the amount of locomotion on the test day as a factor contributing to *Arc* induction; these animals were allowed to locomote in the activity chamber only until they matched the average distance traveled by the unpaired group. Forty-five minutes after the start of exposure, all animals were euthanized for Arc immunohistochemistry. The paired and activity-yoked paired groups did not differ in their Arc levels across brain regions, and both groups had greater levels of Arc expression in regions of the nucleus accumbens, amygdala, and frontal cortex, compared to the unpaired control group. Thus, elevated Arc expression depends upon the association between the activity chamber and cocaine administration. Overlapping corticolimbic regions of enhanced Arc expression after retrieval of a cocaine-context memory (established using either the CPP or CA model) likely signify that these regions (i) are activated during these memories irrespective of preference-based decisions, and (ii) undergo neuroplasticity in order to update information about cues previously associated with cocaine.

Disclosures: Y. Alaghband: None. **J.F. Guzowski:** None. **J.F. Marshall:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 256.20/JJ15

Topic: C.18. Drugs of Abuse and Addiction

Support: NIDA Grant DA029127

Title: Impulsive behavior induced by cocaine is ameliorated by the alpha2A noradrenergic agonist guanfacine in animals performing the 5-choice serial reaction time task

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Abstract: Increases in impulsivity are clinical symptoms associated with the development and maintenance of drug abuse and considered a major risk factor for drug relapse. Similar inhibitory control issues are experienced by individuals diagnosed with attention deficit hyperactivity disorder (ADHD) and medications successfully used to treat this clinical population may have beneficial effects in substance abuse individuals. One such drug successfully used in the treatment of ADHD is the noradrenergic (NA) alpha2A agonist guanfacine which has a good safety profile and low abuse potential. Additionally, given the importance of the noradrenergic system in drug abuse, drugs acting at this neurotransmitter site represent a promising therapeutic strategy. In the present study, we evaluated the effects of guanfacine (0.1-1 mg/kg, ip) in

combination with cocaine (15 mg/kg, ip) using the 5-choice serial reaction time task (5-CSRTT). Male Wistar rats were trained to stable 80% accuracy levels using a standard protocol of 1 sec stimulus duration and 5 sec limited response hold. Administration of cocaine (3.5-15 mg/kg, ip) dose-dependently increased inhibitory control measures (premature and time out responding) with a dose of 15 mg/kg producing the greatest effect on impulsivity. Guanfacine (0.1-1 mg/kg, ip) in combination with cocaine (15 mg/kg, ip) dose-dependently and significantly reduced the impulsivity measures (premature and timeout responding) without affecting task accuracy. Despite these positive effects, some of the behavioral task indices (i.e., omissions and latency to retrieve reward) were slightly altered in comparison to cocaine performance, but not to vehicle performance. These data (in a rodent model) suggest that novel noradrenergic alpha2 agonists (like guanfacine) may have therapeutic potential for controlling impulsive behavior (and possibly, drug replase) in individuals suffering from drug addiction.

Disclosures: P.M. Callahan: None. **R. Schade:** None. **A.V. Terry:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

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Program#/Poster#: 256.21/JJ16

Topic: C.18. Drugs of Abuse and Addiction

Support: CAPES

Title: Neuropsychological evaluation of attention and working memory in addicts submitted to psychotherapy and pharmacotherapy

Authors: *J. E. PANDOSSIO, S. C. MARQUES;
Univ. of Brasilia, Brasília, Brazil

Abstract: Chemical dependency (CD) is characterized by a cluster of symptoms related to maladjusted substance use. The Psychosocial Care Center for Alcohol and Drugs (CAPSad) is a reference to the treatment of CD. This study evaluated the influence of psychotherapy and pharmacotherapy on executive functions such as attention and working memory of addicts in treatment on CAPSad of Ceilandia (DF, Brazil), through psychometric scale (WAIS III - Digits and Sequence of Numbers and Letters subtests). This study also compared the performance of addicts and individuals without history of CD (control group) on the same functions. On this study, there were 60 participants, 30 addicts in the experimental group and 30 in the control group. The experimental group was divided in two, according to the treatment: addicts submitted to the association of psychotherapy and pharmacotherapy (n=21) and only psychotherapy (n=9).

This methodological schedule is in accordance with local and international ethical parameters. The results for the subtests weighted score indicated that the addicts showed no losses in these functions, according to independent t test (t=3.17, p=0.02). However, the experimental performance (M=9.20, SEM=0.48) was found below the control (M=11.03, SEM=0.30), suggesting impairments in cognitive functioning due to drug use history. The results showed no difference between the combination of pharmacotherapy and psychotherapy (M=11, SEM=0.88) and the control - no treatment (M=12.73, SEM=0.33) but, compared to only psychotherapy (M=9.67, SEM=0.80), there was a difference, according to ANOVA One-Way, followed by Tukey test (F(2,46)=6.51, p=0.007). The findings of this study also indicated that the specific interaction of benzodiazepines and anticonvulsants with naltrexone (n=6, M=4.83, SEM=0.60) or with selective serotonin reuptake inhibitors (n=5, M=4.40, SEM=0.60), used in pharmacotherapy of some participants, improved the addicts performance in working memory subtests, compared to only psychotherapy (M=3.88, SEM=0.51), according to ANOVA One-Way, followed by Tukey test (F(3,59)=5.10, p=0.01), similar to the combination of pharmacotherapy and psychotherapy. Taking together, these results confirm that the best treatment strategy for CD is the combination of pharmacotherapy and psychotherapy, focusing on executive functions.

Disclosures: J.E. Pandossio: None. **S.C. Marques:** None.

Poster

256. **"Learning, Memory, Dependence, and Addiction"**

Location: Halls B-H

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Topic: C.18. Drugs of Abuse and Addiction

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Memory-enhancing function of drug reinforcers: A solution to the cocaine puzzle?

Authors: *F. LERI[1], N. RKIEH[2], B. WINTERS[2], J. CLOKE[2];
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Abstract: It has been proposed that the reinforcing properties of drugs of abuse are due, in part, to their ability to enhance memory formation. This hypothesis is primarily supported by evidence of enhanced recall in animals and humans treated with d-amphetamine immediately after training on memory tasks. The aim of the current study was to test this hypothesis using cocaine, a drug that clearly reinforces behavior in many species.
Cocaine (1 - 20 mg/kg, IP) was administered to male Sprague-Dawley rats immediately after

training on “win-stay” tasks in an automated eight-arm radial maze. In win-stay, animals are reinforced with sucrose for entering one specific arm that is illuminated by a cue light. In Experiment 1, post-training cocaine dose-dependently impaired acquisition. The impairment, however, was attributed to an interference with performance caused by some acute side effect of cocaine (i.e., anxiety and/or taste avoidance). In Experiments 2 & 3, rats received a sensitizing regimen of cocaine exposure prior to administration of cocaine during learning of different versions of the win-stay task. Although cocaine sensitization attenuated performance impairments, no clear memory enhancing effect was observed. In Experiment 4, co-administration of diazepam (2 mg/kg) also failed to reveal cocaine-induced memory enhancement. As a matter of fact, diazepam augmented the performance deficit caused by cocaine, and probe tests attributed the deficit to impaired motivation. Finally, Experiment 5 tested the effect of post-training cocaine on a spontaneous object recognition task. In this task, rats are required to discriminate novel from familiar objects and, importantly, performance is not driven by sucrose. Here, post-training cocaine administration caused a clear dose-dependent enhancement of retention.
Taken together, these data suggest that post-training cocaine administration can alter performance on memory tasks by a process that is independent from memory consolidation. The process appears to be associative, and to involve a link between the stimulus that motivates task performance (i.e., sucrose in the win-stay task) and acute side effects of the drug. In tasks were the nature of the stimulus is different (i.e., novelty in object recognition), the memory enhancing effects of cocaine are revealed.

Disclosures: F. Leri: None. **N. Rkieh:** None. **B. Winters:** None. **J. Cloke:** None.

Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Topic: C.18. Drugs of Abuse and Addiction

Support: NIH-NIDA 1R01DA029147

NIH-NIDA DA05130

Title: Self administration of oxycodone by adolescent and adult mice differentially affects gene expressions in hippocampus

Authors: *Y. ZHANG[1], A. J. BROWNSTEIN[1], K. NIIKURA[1], S. D. SCHLUSSMAN[1], A. HO[1], J. OTT[2], M. KREEK[1];

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Abstract: Prescription opioid abuse in adolescence is a pressing public health issue. We aimed to determine whether oxycodone self-administration differentially affects gene expression in the hippocampus of adolescent compared to adult C57BL/6J mice. Groups of adolescent mice (4 weeks old, n=12) and of adult mice (11 weeks old, n=11) underwent surgery during which a catheter was implanted into their jugular veins. Mice were placed in self-administration chambers for acquisition of oxycodone self-administration or were yoked saline control after recovering from surgery. Mice self-administered oxycodone (0.25mg/kg/infusion) 2 hrs/day for 14 consecutive days. Mice were sacrificed within one hour after the last self-administration session and the hippocampus was isolated for mRNA analysis. Gene expression was analyzed with real time PCR using a commercially available PCR array. We found that adolescent mice self-administered less oxycodone than adult mice over the 14 days. Adolescents and adults significantly differed in Calcium/calmodulin-dependent protein kinase, Glutamate receptor, AMPA2 and metabotropic 5 mRNA levels in the absence of oxycodone exposure. 14-day oxycodone self administration increased Proviral integration site 1 and Thymoma viral proto-oncogene 1 mRNA levels compared to controls in both adults and adolescents. More genes in the hippocampus of adult changed in response to oxycodone self administration compared to controls than in adolescent mice. Overall, this study demonstrates for the first time that repeated oxycodone self-administration differentially altered gene expression in the hippocampus of adolescent versus adult mice.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: C.18. Drugs of Abuse and Addiction

Support: Department of Veterans Affairs

Title: Extinction of opiate reward reduces dendritic arborization and dendritic spines in the nucleus accumbens core but not shell

Authors: *G. B. KAPLAN[1], K. A. LEITE-MORRIS[1], K. L. KOBRIN[1], S. C. HEINRICHS[2], O. A. MOODY[2];

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Abstract: Recurrent opiate administration paired with environmental cues leads to conditioned drug reward and cue-induced drug craving and relapse. This study characterizes structural plasticity associated with extinction of conditioned opiate reward. We modeled the morphine reward process in C57BL/6 mice using acquisition and extinction of conditioned place preference (CPP). Extinction learning reduces conditioned responding to drug cues by creating a new memory associated with changes in neuroplasticity. In this study, changes in dendritic plasticity were examined in the nucleus accumbens (NAc) of morphine CPP and extinction-trained mice. Mice were trained to associate morphine injection with a paired chamber. Mice conditioned with different doses of morphine (1, 2.5, 5, and 10 mg/kg, SC) acquired dose-dependent CPP after four morphine exposures as evidenced by difference scores that were significantly higher than saline controls. Other mice were divided into three groups: morphine CPP (10 mg/kg) followed by extinction training, morphine CPP followed by sham extinction training, and saline controls. After acquisition of CPP, mice underwent three extinction training sessions, Extinction I (Days 11-14), Extinction II (Days 16-19), and Extinction III (Days 21-24). Mice underwent Post-Extinction or Post-Sham Extinction training preference tests on days 15, 20, and 25 and then on day 25 brains were used for Golgi-Cox staining. Mice extinguished their preference for the morphine associated compartment within the first extinction session consisting of two paired chamber exposures. Medium spiny neurons from mice were traced for dendritic length, dendritic count, dendritic intersections, dendritic spine density, and spine numbers. Mice in the morphine CPP extinction group (compared to morphine CPP sham extinction group) showed a decreased distribution of spines per neuron in the nucleus accumbens core but not shell. Morphine CPP extinction also reduced the number of dendritic intersections in the core but not shell as demonstrated by Scholl analyses. The extinction group also had decreased total dendritic length per neuron and total dendritic count per neuron in comparison to the other groups. These findings are consistent with others demonstrating that repeated exposure to opiates reduces dendritic arborization and spines in the NAc. Such dendritic changes regulate the the strength of synaptic transmission and may mediate extinction learning relevant to opiate addiction.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

Location: Halls B-H

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Program#/Poster#: 257.03/KK2

Topic: C.18. Drugs of Abuse and Addiction

Title: Prior morphine exposure blocks oxytocin excitation of nucleus accumbens shell neurons

Authors: *M. MOADDAB[1,2], B. HYLAND[2], C. BROWN[1,2];
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Abstract:
Drug addiction is a chronically relapsing disorder that involves compulsive and uncontrolled drug-seeking and drug-taking. The nucleus accumbens (NAc) shell is particularly associated with reward-related behaviours, including those associated with drugs of abuse. Recently, the neuropeptide, oxytocin, has been shown to modulate reward-related behavioural responses to drugs of abuse. Hypothalamic oxytocin neurons project to many forebrain regions, including the NAc shell. We have found that intracerebroventricular (ICV) oxytocin administration enhances the expression of morphine-induced conditioned place preference (CPP). So, we used in vivo single-unit extracellular recording in urethane-anesthetized male Wistar rats to investigate the effects of ICV oxytocin on the firing rate of NAc shell neurons in morphine-naive rats and morphine-treated rats (three daily injections of morphine, with the last injection 24 h before recording). In morphine-naive rats, ICV saline (2 µl) administration did not change the firing rate of NAc shell neurons (1.3 ± 0.2 to 1.2 ± 0.3 spikes s^{-1}, n = 29; $P = 0.74$, one-way repeated measures ANOVA), whereas ICV oxytocin (0.2µg/2µl) increased NAc shell neuron firing rate (2.4 ± 0.6 to 3.2 ± 0.8 spikes s^{-1}, n = 29; $P < 0.01$). The basal firing rate of NAc shell neurons in morphine-treated rats (1.1 ± 0.2 spikes s^{-1}, n = 70) was similar to that in morphine-naive rats (1.8 ± 0.3 spikes s^{-1}, n = 80; $P = 0.06$, unpaired *t*-test). In morphine-treated rats, neither saline (1.0 ± 0.2 to 1.2 ± 0.2 spikes s^{-1}, n = 23; $P = 0.58$) nor oxytocin (1.7 ± 0.5 to 1.6 ± 0.4 spikes s^{-1}, n = 21; $P = 0.56$) affected the firing rate of NAc shell neurons. These results show that three daily injections of morphine block the excitation of NAc shell neurons by subsequent ICV oxytocin, suggesting that morphine-induced adaptations reduce the excitability of NAc shell neurons to oxytocin administration. Hence, the oxytocin enhancement of the expression of morphine-induced CPP on the test day appears unlikely to be due to acute modulation of the baseline firing rates of NAc shell neurons. Rather, morphine-induced adaptation, including the abolition of the NAc response to oxytocin, might enable oxytocin-induced modulation of other neuronal circuits to influence the expression of morphine-induced CPP.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

Location: Halls B-H

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Program#/Poster#: 257.04/KK3

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH-NIAAA#16658

Title: Mapping the brain functional and structural connectivity of mu-opioid receptor knock-out mice

Authors: *A. MECHLING[1,2,4], T. AREFIN[1,4,3], H.-L. LEE[1], M. REISERT[1], S. BEN HAMIDA[4], J. HENNIG[1], D. VON ELVERFELDT[1], B. KIEFFER[4], L.-A. HARSAN[1];
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Abstract: A non-invasive insight into the brain's intrinsic connectional architecture of functional networks (FN) has only become possible since the development of resting-state functional magnetic resonance imaging (rsfMRI). In humans, the default mode functional networks and their alterations in pathologies are intensively studied. Moreover, when combined with diffusion tensor magnetic resonance imaging (DT-MRI) and fiber tracking investigations [1], recent studies demonstrate the structural connectivity features underlying the FN and their remodeling mapped by rsfMRI [2]. However, the intrinsic connectional architecture of functional and structural networks in the mouse brain remains a significantly underexplored research area. The goal of the present study was to bridge this gap by unifying and adapting the rsfMRI/DT-MRI techniques for studying the functional and structural connectivity pattern in mouse models of brain disorders. We focused our investigation on mapping the brain connectional networks of mu-opioid receptor (MOR) knock-out mice (Oprm1-/-), an extensively used model of drug addiction and reward [3].
Mouse brain MRI was performed with a 7T small bore animal scanner and a mouse head adapted cryogenic surface coil (Bruker Germany) both allowing for high signal-to-noise ratio and short acquisition times at high resolution. rsfMRI and DT-MRI data was acquired from 8 weeks old wild type (n=14) and Oprm1-/- (n=14) male mice using single shot Gradient Echo Echo Planar Imaging (GE-EPI) and 4 shot DT-EPI sequences. Group Independent Component Analysis (ICA) of rsfMRI data allowed the identification of elementary functional clusters of the Oprm1-/- mouse brain. Their connectional relationship was tested with partial correlation and graph theory providing a comprehensive picture of Oprm1-/- brain functional connectivity. As a step forward, the identified functional clusters were subsequently used as regions of interest in a fiber tracking algorithm, for mapping structural connectivity. We focused our analysis on brain

networks involving areas known for their clustered expression of MOR such as striatum, amygdala and thalamus. Our experiment broadens the knowledge about functional and anatomical connectivity in mouse models of brain disorders uncovering also the involvement of the mu opioid receptor in brain networks remodeling. This non-invasive study design forms also the basis for longitudinal investigations, opening a perspective towards testing therapeutic compounds and their influence on the progress of disease patterns.
[1] Harsan et al, PNAS 2013; [2] van den Heuvel et al, HBM 30, 2009; [3] Kieffer et al, ProgNeurobiol 66, 2002

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Topic: C.18. Drugs of Abuse and Addiction

Support: 1R21DA030225

Title: Novel mechanism for opioid drug action: Implications of Redox/Methylation signaling

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Abstract: Drugs of abuse, including opioids, act upon molecular mechanisms which support attention, awareness and consciousness. Attention is closely linked to learning and memory, & frequent drug use results in persistent behavioral changes, including withdrawal syndromes, indicating that neuronal systems have adapted to repeated drug use. Mounting evidence indicates that epigenetic changes, specifically alterations in patterns of DNA and histone methylation, can produce long lasting alterations in gene expression, affecting learning, memory and behavior. Thus, the activities of methylation-related metabolic pathways in neuronal cells could help explain at least some molecular aspects of the acute and longer-term effects of opioid class of drugs of abuse. We investigated acute and long-term effects of selected opioid drugs and their mechanism of influence on pathways of sulfur metabolism, redox and DNA methylation status in cultured neuronal cells. We found that morphine dose dependently inhibited EAAT3, which transports cysteine, a precursor for glutathione (GSH) synthesis. Subsequent decreases in

GSH/GSSG and SAM/SAH ratios were also observed with prolonged morphine treatment. Mechanistic investigation suggested involvement of PI3-Kinase and Protein Kinase A (PKA) for short-term, and MAP-Kinase signaling pathways for long-term inhibition of cysteine uptake. Global DNA methylation, measured as an index of 5-methylcytosine levels, correlated to the observed changes in SAM/SAH ratio. To further confirm these observed results, qRT-PCR analysis was performed for mRNA levels of enzymes and transporters involved in antioxidant and methylation pathways. In particular, an increase in mRNA levels of LINE-1 retrotransposon, induced by prolonged morphine treatment was observed, indicating a decrease in global DNA methylation. Lastly, redox and methylation responses were also investigated upon removal of morphine after a prolonged exposure (i.e. in vitro wash out). There was a prompt increase in cysteine uptake after in-vitro washout. Similarly, intracellular GSH and cysteine were also increased. Corresponding changes were also observed in global DNA methylation.These results indicate a novel epigenetic signaling pathway for opioid drugs, involving changes in cellular redox status and methylation capacity, mediated by multiple signaling pathways. These findings have implications for drug tolerance and withdrawal, and may lead to development of novel therapeutic interventions. Additionally, this redox-methylation signaling pathway might also be implicated as a mechanism for other classes of drugs of abuse, such as amphetamines and alcohol.

Disclosures: M.S. Trivedi: None. **N. Hodgson:** None. **J. Shah:** None. **R. Deth:** None.

Poster

257. **Opioids: Neural Mechanisms of Addiction**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 257.06/KK5

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Title: Opiate exposure state controls a novel, CaMKIIα/ ERK1/2-dependent molecular opiate reward memory switch in the basolateral amygdala-prefrontal cortical pathway

Authors: *L. G. ROSEN[1,2], W. J. RUSHLOW[1,2,3], S. R. LAVIOLETTE[1,2,3];
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Abstract: The potent rewarding effects of opiate class drugs facilitate the formation of strong associative memories linked to the drug experience and play a key role in triggering relapse. The basolateral amygdala (BLA) is involved in the encoding of acute opiate reward memories. We have reported recently that the localization of associative opiate memories anatomically and temporally shifts from the BLA to the medial prefrontal cortex (mPFC) for long-term storage between these two interconnected areas (Gholizadeh et al., 2013). Interestingly, intra-BLA processing of opiate-related reward memories is independently controlled by dopamine D1 and D2 receptor signaling as a function of opiate exposure state. Transmission via D1 receptors is required for acute opiate memory formation in the previously drug-naïve state, whereas D2-mediated signaling is implicated in memory formation following the transition to opiate dependence and withdrawal. However, the underlying molecular substrates controlling memory formation within the BLA>mPFC pathway are not presently understood. Here, we show that opiate reward memories are processed via an ERK-dependent, D1-mediated mechanism in the naïve state, but through a CaMKIIα-dependent, D2 mediated mechanism in the dependent/withdrawn state. Using an unbiased place conditioning procedure combined with protein analyses of BLA and mPFC tissue, we report that intra-BLA associative opiate-related memory formation depends upon ERK1/2 signaling in the drug-naïve state, but switches to a CaMKIIα-dependent signaling substrate in the dependent/withdrawn exposure state. Consistent with our behavioural findings, BLA western blotting protein analyses demonstrated a reduction in both phosphorylated ERK1/2 as well as total and phosphorylated CaMKIIα in opiate dependent/withdrawn animals. Interestingly, protein analysis in the mPFC has revealed a functionally opposite pattern of ERK1/2 and CaMKIIα protein expression, with an increase in both CaMKIIα and ERK1/2 in the dependent/withdrawn state, demonstrating a functional dissociation in molecular memory pathways across the BLA and mPFC controlled by opiate exposure state and withdrawal. Our results further demonstrate a functional interaction between the BLA and mPFC during the processing and storage of opiate-related associative memories and the switch from the non-dependent, to dependent/withdrawn opiate states. Using an unbiased conditioned place preference paradigm paired with local microinfusions of ERK1/2 and CaMKIIα inhibitors into the mPFC, the behavioural significance of these molecular changes are currently being examined and will be presented.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Topic: C.18. Drugs of Abuse and Addiction

Support: NRSF2007-2013 ARISTEIA 1361

Title: A role of Regulator of G protein signaling 9-2 (RGS9-2) in the Nucleus Accumbens in opiate addiction and analgesia

Authors: *S. GASPARI[1], M. PAPACHATZAKI[1], M. E. TSIMBANOULI[1], K. DEISSEROTH[2], M. LOBO[3], V. ZACHARIOU[4];
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Abstract: Regulator of G Protein Signaling 9-2 (RGS9-2) is a multifunctional signal transduction protein with high levels of expression in the striatum. RGS9-2 plays a major role in the modulation of several GPCR responses in the striatum, and has been shown to potently modulate dopaminergic and opioidergic transmission. We recently identified RGS9-2 complexes in the striatum associated with the acute and chronic actions of different mu opioid receptor agonists. We have also developed viral mediated gene transfer approaches to investigate the actions of RGS9-2 in particular brain regions of adult animals. Using adenoassociated viruses (AAV) expressing RGS9-2 or a dominant negative form of the protein (DEPless RGS9-2) we explored the brain specific actions of this molecule in a series of behavioral paradigms for opiate reward and analgesia. Our studies reveal that RGS9-2 actions in the nucleus accumbens (NAc) negatively regulate the rewarding actions of morphine and they also affect the development of physical dependence. In addition, RGS9-2 in the NAc plays a key role in morphine analgesia and tolerance in paradigms of acute and chronic pain. These findings are further supported by optogenetic studies, in which subpopulations of dopamine D1 or dopamine D2 receptor enriched neurons were activated following morphine administration. Activation of D1 receptor enriched neurons leads to an increase in RGS9-2 levels and accelerates the development of analgesic tolerance. These findings point to RGS9-2 complexes in the NAc as novel targets for the treatment of addiction and reveal the influence of RGS9-2 in the NAc in morphine analgesia and tolerance.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Support: U.S. Dept. of Veterans Affairs Biomedical Laboratory Research & Development Service I01BX000218 to KDB and the Stress and Motivated Behavior Institute

Title: Exploring morphine reward and changes in opiate-related signaling molecules in the Wistar Kyoto rat

Authors: *T. S. DENNIS[1], K. D. BECK[2], T. P. COMINSKI[2], S. A. MORRIS BOBZEAN[1], R. J. SERVATIUS[2], L. I. PERROTTI[1];
[1]Psychology, Univ. of Texas at Arlington, Arlington, TX; [2]Neurol. and Neurosciences, Stress and Motivated Behavior Inst., UMDNJ, Newark, NJ

Abstract: The Wistar-Kyoto (WKY) rat has been proposed as a model of anxiety vulnerability as it exhibits pronounced behavioral inhibition, passive avoidance, exaggerated startle response, enhanced HPA axis activation, and active avoidance that is resistant to extinction. Accumulating evidence suggests that WKY rats respond differently to rewarding stimuli when compared to outbred strains of rat. A recent study from our laboratory shows that WKY rats are more sensitive to higher doses of cocaine than Sprague Dawley (SD) rats. The present study was designed to extend our previous findings and characterize morphine conditioned place preference (CPP) in WKY rats. The equipment used to quantify CPP consisted of a three-chambered apparatus. Two visually and contextually distinct compartments are separated by a small shuttle compartment. Fifteen infrared photobeam detectors were used for automated data collection. Adult male WKY and SD rats were subjected to a CPP paradigm, which included a preconditioning test, a conditioning phase, and an acquisition test. The preconditioning test was performed to identify any initial bias for either side of the CPP apparatus. All rats were conditioned with one of six doses of morphine (0, 0.5, 1.25, 2.5, 5, or 7.5.mg/kg) and saline on alternating days for a total of six conditioning days. The acquisition test began 24 hours after the last conditioning session. In this test, rats were allowed free access to all chambers for 30 minutes in a drug-free state. The increase in time spent in the morphine-associated compartment was considered a measure of conditioned preference. SD rats displayed morphine-induced CPP to each of the five doses of morphine tested. The WKY rats displayed a preference only at the 1.25, 2.5, and 5mg/kg doses but showed an aversion at the 7.5mg/kg dose. This study shows that WKY rats display a greater sensitivity to higher doses of morphine. While it is clear the WKY rat has a distinctive response to drugs of abuse, more research is needed to fully elucidate the mechanisms that drive this unique behavioral profile. Studies are currently underway to further characterize alterations in the reward neurocircuitry of these rats.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Topic: C.18. Drugs of Abuse and Addiction

Support: R01 DA025036

Title: Enhanced protein phosphatase 2A (PP2A) activity drives SK2 channel mediated impairment of long term potentiation (LTP) in the hippocampus after context-dependent morphine

Authors: *A. K. FAKIRA[1], G. S. PROTUGAL[1], Z. MELYAN[1], D. S. SULZER[2], J. A. MORON CONCEPCION[1];
[1]Anesthesiol., [2]Neurol., Columbia Univ. Med. Ctr., New York, NY

Abstract: Learned associations between drugs of abuse and context can have powerful effects on drug craving and relapse. Context-dependent locomotor sensitization and conditioned place preference (CPP) occur with escalating doses of morphine paired with a novel context and are associated with impaired long term LTP in the hippocampus. Our studies demonstrate that Ca^{2+} permeable glutamate receptors, AMPA and NMDA, are inserted into the synapse after morphine treatment. Small conductance Ca^{2+} activated potassium type 2 (SK2) channels have been shown to modulate LTP. Dephosphorylation by PP2A increases Ca^{2+} sensitivity of SK2 thus increasing the channel function. Since Ca^{2+} is known to increase PP2A activity Ca^{2+} influx from AMPA and NMDARs may activate PP2A and potentiate SK2 channel function. Our studies show that mice that display locomotor sensitization have increased SK2 channel function and PP2A activity. Ultimately, inhibition of either PP2A and SK2 channels restores LTP to normal levels. Future studies will determine if these mechanisms are involved in LTP impairment following morphine CPP. Additionally, using 2-photon imaging of dendritic spines we plan on investigating if pairing morphine with a novel context results in increases in AMPA and NMDAR-mediated Ca^{2+} transients in the spine. . Altogether, our data highlight new mechanisms underlying context-dependent drug seeking behavior and may lead to development new therapeutic approaches to preventing relapse.
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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Topic: C.18. Drugs of Abuse and Addiction

Support: NSERC

Brain Canada

Title: Orexin/hypocretin in the ventral tegmental area is necessary for morphine-induced synaptic plasticity of dopamine neurons

Authors: *C. BAIMEL[1], S. L. BORGLAND[2];
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Abstract: Dopamine neurons in the ventral tegmental area (VTA) are a key target of addictive drugs and neuroplasticity in this region may underlie some of the core features of addiction. All drugs of abuse induce an LTP-like potentiation of excitatory inputs to VTA dopamine neurons, but it is not well understood how these drugs, despite chemical diversity and differences in molecular targets, bring about a common synaptic change. Orexin (hypocretin) is a lateral hypothalamic neuropeptide released into the VTA that promotes drug-seeking behaviours and potentiates excitatory synaptic transmission in the VTA. Inhibition of systemic orexin signaling blocks both cocaine-induced plasticity and drug seeking behaviours. However, it is unknown how orexin gates drug-induced plasticity in the VTA. We determined if orexin action in the VTA was necessary for morphine-induced strengthening of excitatory synapses onto VTA dopamine neurons. We used whole cell patch clamp electrophysiology in midbrain slices taken from male Sprague Dawley rats 24 hours following a single in vivo injection of morphine (10 mg/kg) or saline to assess excitatory synaptic strength. Morphine potentiated glutamatergic synaptic transmission onto VTA dopamine neurons by a presynaptic increase in glutamate release and by a postsynaptic change in AMPAR number or function, likely including a switch in subunit composition. Systemic or intra-VTA administration of SB 334867, an orexin receptor type 1 antagonist, blocked a morphine- induced increase in the AMPAR/NMDAR ratio, morphine-induced increases in AMPAR mini excitatory postsynaptic current frequency and amplitude, as well as morphine-induced AMPAR subunit redistribution measured by a change in rectification. These results support a role for orexin signaling in both pre-and postsynaptic potentiation of glutamatergic transmission in the VTA by morphine. Because orexin signaling is required for

plasticity induced by both morphine and cocaine, orexin may function as a gatekeeper for drug-induced plasticity of glutamatergic inputs to dopamine neurons.

Disclosures: C. Baimel: None. **S.L. Borgland:** None.

Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Topic: C.18. Drugs of Abuse and Addiction

Support: MIUR

Fondazione Sanpaolo

Title: Chronic morphine and morphine withdrawal dynamically regulate the rpS6 multiple phosphorylation in a brain region-specific manner

Authors: A. CICCARELLI[1], A. CALZA[2], F. SANTORU[2], A. CONCAS[2], *M. SASSOE-POGNETTO[1], M. GIUSTETTO[1];
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Abstract: The activation of the extracellular regulated kinase (ERK) is a necessary step for several cognitive processes such as learning and memory formation, and is also involved in the onset of addiction caused by drugs of abuse. In the context of opiate abuse, it is still unclear how ERK signaling is recruited by morphine administration and what are its downstream effectors. One target of ERK activation is the ribosomal protein S6 (rpS6), a component of the 40S ribosomal subunit whose phosphorylation levels correlate with the rates of protein synthesis and that is regulated also by the AKT/mTOR pathway. In a previous study we showed that while ERK signaling is down-regulated in neurons of the nucleus accumbens (NAc) and lateral septum (LS) after chronic morphine treatment, naloxone-precipitated withdrawal activated ERK pathway in these brain areas. In this work we found that chronic morphine produces a decrease of the number of neurons showing phospho(p)-rpS6 immunoreactivity (both for ser 230/235 and 240/244 sites) in the LS, while an increase was found in the dorsomedial striatum. Interestingly, naloxone-precipitated morphine withdrawal produced a robust increase of rpS6 phosphorylation both in the LS and the NAc, as well as in the somatosensory cortex. Moreover, the analysis of double labeling experiments revealed that phospho-ERK and p-rpS6 are co-localized in LS neurons of withdrawal rats. These data underscore the importance of rpS6 in the modulation of

morphine effects in the brain and suggest that withdrawal may produce its negative effects through an increase of translational activity mediated by the activation of both ERK and mTOR pathways. Moreover, the heterogeneous and dynamic activation of phospho-rpS6 unveils a complex and parallel activation of multiple brain regions following opioid treatment.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

Location: Halls B-H

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Topic: C.18. Drugs of Abuse and Addiction

Title: Differential effects of hydrocodone, oxycodone, and morphine on the response of D2/D3 dopamine receptors in adolescent mice

Authors: *M. A. EMERY, M. L. S. BATES, P. J. WELLMAN, S. EITAN;
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Abstract: Oxycodone and hydrocodone are prescribed analgesics for children and adolescents. In addition, these prescription opioids are frequently abused among adolescents. The adolescent brain is still developing, thus exposure to opioids in both pain relief and abuse contexts might have different long term outcomes in this population than in adults.
Our recent studies demonstrate that morphine exposure differentially modulates the dopaminergic system in adolescent and adult mice. We observed that adolescent mice exposed to morphine exhibited behavioral supersensitivity of the D2Long/D3 postsynaptic receptors. This effect of morphine was extremely pronounced in adolescents but was hardly observed in adults. We have also demonstrated that buprenorphine and methadone, opioids used for maintenance treatment, differentially alter the responses of the dopaminergic system in adolescents. In the current study, we compared hydrocodone, oxycodone, and morphine for their effect on D2L/D3 receptor activity.
Adolescent mice were orally administered oxycodone, hydrocodone, morphine, or saline for 6 days. Twenty four hours after the last opioid dose, mice were examined for their locomotor response to quinpirole, a D2/D3 receptor agonist. Additionally, the effect of exposure to the various opioids on modulating D2 receptor-induced signaling molecules was explored using Western blot.
Quinpirole (10 mg/kg) reduced locomotor activity in drug-naïve adolescent mice, in line with the

literature demonstrating that the predominant effect of quinpirole on motor activity is suppressive in mice. Consistent with our previous findings, in the opioid-treated adolescent mice, initial suppression of locomotor activity was observed, but was followed by enhanced locomotion. However, the extent of this effect differed across the various opioids. The most profound effect was observed after exposure to oxycodone, followed by significantly less effect of hydrocodone. The least effect was observed following exposure to morphine. Additionally, differential effects on downstream signaling molecules were observed following exposure to the various opioids.
These findings suggest that exposure to various opioids carry differential risks in altering the highly sensitive neurochemistry of adolescents' brain, especially in regards to the D2L receptor system. These results call for more research to reveal individual differences between different adolescents recreationally using (misusing/abusing) various opioids. Additionally, these studies strongly indicate potential age-dependent and drug-dependent risks in administering opioids to adolescents for pain management.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Topic: C.18. Drugs of Abuse and Addiction

Support: DA004043

Title: Hypocretin-2 receptor antagonism dose-dependently reduces compulsive-like self-administration of heroin in rats allowed extended access

Authors: *B. E. SCHMEICHEL[1], L. F. VENDRUSCOLO[1], K. K. MISRA[1], J. E. SCHLOSBURG[1], C. CONTET[1], D. E. GRIGORIADIS[2], G. F. KOOB[1];
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Abstract: Animal models of drug dependence indicate that extended access to the drug produces escalation of intake over time, compared with stable levels of intake observed in animals maintained on a limited access schedule. Importantly, escalation of drug intake has been suggested to model the transition from controlled drug use to compulsive-like drug seeking/taking. Recently, the hypocretin/orexin (HCRT) neuropeptide has been associated with

both drug reinforcement and reinstatement, implicating HCRT-1 receptor signaling, in particular, in drug-related behaviors for all major drug classes, including psychostimulants, nicotine, alcohol and opiates. However, to date there are limited studies investigating the role of HCRT-2 receptor signaling in drug seeking, especially under extended access self-administration conditions. The current study examined the effects of administering the HCRT-2R antagonist, NBI-80713, on heroin self-administration in rats allowed short- (1 hour; ShA) or long- (12 hour; LgA) access to intravenous heroin self-administration. Results indicate that systemically administered NBI-80713 dose-dependently decreased heroin self-administration in LgA, but not ShA, animals. These observations suggest a functional role for HCRT-2 receptor signaling in compulsive-like heroin self-administration with extended access and indicate HCRT-2R antagonism as a potential pharmacological target for the treatment of heroin dependence.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Title: Enhanced heroin self-administration is associated with deficit of CREM in NAc core of an animal model of impulsivity

Authors: *Y. REN, C. V. MORRIS, H. SZUTORISZ, Y. L. HURD;
Neurosci. & Psychiatry, The Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: There is a strong association between trait impulsivity and addiction in humans. However, the neurobiology of impulsivity and its relationship to addiction vulnerability is poorly understood. This experiment was designed to study a common molecular basis between impulsivity and addiction vulnerability. Heroin self-administration (SA) was studied in spontaneously hypertensive rats (SHR), an impulsive animal model, with Wistar Kyoto rats

(WKY) as a control. In vivo dopamine in NAc core was measured using microdialysis. Expressions of genes of DA system as well as synaptic plasticity were studied by a customized PCR array. Compared with WKY, SHR took more heroin during the maintenance, showed higher resistance to extinction and enhanced heroin-seeking upon cue-reinstatement. In drug naive animals, SHR were more impulsive than WKY as characterized by intolerance-to-delay (ITD) task. In vivo DA increased to a greater extent in SHR NAc core in response to an acute heroin, but no differences in basal DA level between two strains. Gene expressions of DA receptors were not different between two strains. However, a significant decrease of CREM gene expression was detected in SHR NAc core but not shell, which was associated with less enrichment of 3meH3K4 and H3Ac at CREM gene. These data suggest that the impulsivity of SHR involves in CREM deficit regulated by histone lysine methylation, which might contribute to their heroin vulnerability.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Support: DA024030

DA027128

Title: Expression of the superoxide generating NADPH oxidase Nox2 gene in the nucleus accumbens is required for morphine conditioned place preference

Authors: *M. J. GLASS[1], E. OGORODNIK[1], P. ZHOU[1], R. L. DAVISSON[2,3], C. IADECOLA[1];
[1]Brain and Mind Res. Inst., [2]Cell and Developmental Biol., Weill Cornell Med. Col., NEW YORK, NY; [3]Col. of Vet. Med., Cornell Univ., Ithaca, NY

Abstract: Although reactive oxygen species (ROS) like superoxide have traditionally been viewed as deleterious byproducts of oxidative metabolism, recent evidence indicates that they can also act as important signaling molecules generated by specific and highly regulated enzymes in the brain. Although ROS have been recently implicated in neural processes critical for addiction, including the modulation of glutamate signaling, synaptic plasticity, and learning and memory, there is little direct evidence that specific ROS producing enzymes are involved in

opioid addictive behaviors. We provide evidence that the superoxide-generating NADPH oxidase (NOX) plays a role in the expression of classically conditioned behaviors associated with opiate exposure. Mice with a constitutive knockout (cKO) of the catalytic Nox2 NOX subunit showed deficits in a morphine (10 mg/kg) conditioned place preference (post-training difference scores (sec): +160±40 versus +50±30, $p<0.05$, wild-type compared to Nox2 cKO mice, respectively). The reduction in morphine CPP was not simply the result of a generalized deficit in place learning, since separate groups of wild-type and Nox2 cKO mice made dependent on morphine and trained to express a naloxone withdrawal conditioned place aversion showed comparable aversive learning (wild-type: -230±110 sec versus Nox2 cKO: -210±71 sec, $p>0.8$). Wild-type and Nox2 cKO mice did not differ in morphine analgesia (tail-flick latency (sec) at 52.5º C; wild-type: 5.1±0.6 versus Nox2 cKO: 4.7±0.7, $p>0.1$), indicating that these differences were also unrelated to genotype-dependent effects on morphine pharmacokinetics. Compared to wild type mice, Nox2 cKO's showed reduced immediate early gene expression in the nucleus accumbens (Acb) in response to exposure to a morphine paired environment. Spatial-temporal gene knockdown produced by microinjection of an adenovirus expressing a small interfering Nox2 RNA (siNox2) in the Acb impaired the expression of morphine place preference compared to mice receiving administration of a control (siCnt) vector (siCnt: +245±60 sec versus siNox2: +80±30 sec, $p<0.05$). These results provide the first evidence that Nox2 expression in the Acb plays a critical role in classically conditioned behaviors associated with opiate exposure; this information may enhance our understanding of opiate action by identifying novel free radical mediated intracellular signaling pathways involved in opioid addiction and provide novel targets for the development of future pharmacotherapy.

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Poster

257. **Opioids: Neural Mechanisms of Addiction**

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Support: P50DA011806

Title: Girk3 in the ventral tegmental area (VTA): A key determinant of sensitivity to opioid reward-related behavior

Authors: *L. A. KOTECKI[1], M. B. MUNOZ[2], P. A. SLESINGER[3], K. WICKMAN[1]; [1]Pharmacol., Univ. of Minnesota, Minneapolis, MN; [2]The Salk Inst., La Jolla, CA; [3]Dept. Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The ventral tegmental area (VTA) and interconnected brain regions including the nucleus accumbens and medial prefrontal cortex comprise in part, the mesocorticolimbic dopamine (DA) system. The mesocorticolimbic DA system mediates the reward-related behavioral effects of all drugs of abuse, including opioids. Opioids stimulate dopaminergic neurons in the VTA indirectly, via direct inhibition of GABAergic neurons located in the VTA and/or rostromedial tegmental nucleus (RMTg). Indeed, GABAergic input to VTA DA neurons represents a critical regulator of DA neurotransmission in the reward circuitry. VTA DA neurons express a unique subtype of G protein-gated inwardly-rectifying K+ (Girk/KIR3) channel - a heterotetramer containing Girk2 and Girk3 subunits. The Girk2/Girk3 heterotetramer in VTA DA neurons mediates the postsynaptic inhibitory effect of GABAB receptor stimulation, which potently suppresses VTA DA neuron output. Interestingly, the residual Girk channel in VTA DA neurons from Girk3-/- mice (presumably a Girk2 homotetramer) is more sensitive to GABAB receptor stimulation than the Girk2/Girk3 heterotetramer expressed in VTA DA neurons from wild-type mice. Previous work from our group has identified Girk3 as a key determinant of the sensitivity of mice to the analgesic effects of opioids, cannabinoids, and clonidine. Girk3 has also been implicated in withdrawal from barbiturates, ethanol, and opioids, and the reinforcing effect of cocaine. Here, we show that Girk3-/- mice are less sensitive than wild-type counterparts to the acute motor-stimulatory effect of systemic morphine. Importantly, lentiviral-mediated expression of Girk3 in the ventral midbrain of Girk3-/- mice restored the normal sensitivity of the Girk channel in VTA DA neurons to GABAB receptor stimulation, and the normal sensitivity of Girk3-/- mice to the motor-stimulatory effect of systemic morphine. These data support the contention that increased sensitivity of VTA DA neurons to inhibitory input is a key and perhaps titratable determinant of sensitivity to the reward-related behavioral effects of drugs with abuse potential.

Disclosures: L.A. Kotecki: None. **M.B. Munoz:** None. **P.A. Slesinger:** None. **K. Wickman:** None.

Poster

257. **Opioids: Neural Mechanisms of Addiction**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 257.17/LL8

Topic: C.18. Drugs of Abuse and Addiction

Support: NSERC Grant

Title: Memory consolidation processes engaged by relapse to heroin seeking: Role of noradrenergic locus coeruleus complex

Authors: *E. L. CUMMINS, E. BOUGHNER, J. GRANT, K. KENT, T. MACDONALD, D. KWIATKOWSKI, F. LERI;
Univ. Guelph, Guelph, ON, Canada

Abstract: It is thought that relapse to drug taking involves new learning characterized by memory consolidation. These studies were designed to explore the role of the noradrenergic (NA) cell groups of the locus coeruleus (LC) complex in the memory consolidation process engaged by relapse. To do this, we used a conditioned place preference (CPP) reacquisition model of relapse in male Sprague-Dawley rats. This involved 7 phases: habituation, place conditioning (1 mg/kg heroin or 20 mg/kg cocaine, and vehicle; 4 place pairings each), test of conditioning (Test I), extinction (vehicle only; 4 place pairings each), test of extinction (Test II), reconditioning (heroin or cocaine, and vehicle; single place pairing each) and test of reconditioning (Test III). Rats received systemic (vehicle, 10, 40 or 100 ug/kg) or intra-LC infusions of clonidine (vehicle, 4.5 or 18 nmol), an alpha 2-receptor agonist, following drug reconditioning with the goal of disrupting the memory consolidation process underlying reacquisition of CPP. To establish the role of NA input to the basolateral amygdala (BLA), an LC projection site important for associative memory, on heroin reacquisition, rats received intra-BLA infusions of a prazosin/propranolol mixture (alpha 1-receptor antagonist/non-selective beta receptor antagonist respectively; vehicle or 2.4/34 nmol) after reconditioning. The effect of intra-LC clonidine on cellular activity within the BLA was assessed using an immunohistochemical stain for the immediate early gene c-fos. We found that post-reconditioning, systemic or intra-LC clonidine dose dependently blocked heroin reacquisition when given immediately or 4h after, but had no effect when given 8h after reconditioning (systemic data only) or 4h before Test III. Post-reconditioning clonidine also blocked cocaine reacquisition, suggesting that the effect of clonidine is not due to a diminution of heroin withdrawal symptoms. Further, we show that activation of alpha 1- and beta NA receptors in the BLA is critical for the consolidation of a drug memory, as blocking these receptors also abolished heroin reacquisition (pending BLA histological analysis). We speculate that the disruptive effect of clonidine on memory is at least partially due to reduced NA activity in the BLA. In fact, BLA c-fos activity was dose-dependently reduced following intra-LC clonidine, further relating the essential involvement of LC activation and NA release within the BLA on heroin reacquisition. Together, these data suggest that relapse involves a memory consolidation process sensitive to manipulations of the noradrenergic LC complex.

Disclosures: E.L. Cummins: None. **E. Boughner:** None. **J. Grant:** None. **K. Kent:** None. **T. MacDonald:** None. **D. Kwiatkowski:** None. **F. Leri:** None.

Poster

257. **Opioids: Neural Mechanisms of Addiction**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 257.18/LL9

Topic: C.18. Drugs of Abuse and Addiction

Support: ICyTDF Grant PICD 08-13

CONACyT Grant 82728

Title: Pro-opiomelanocortin mRNA expression is selectively altered by ethanol exposure in the rat brain

Authors: *K. HERNANDEZ FONSECA, E. CABRERA-MUÑOZ, C. REYES, M. MENDEZ; Neurochemistry, Inst. Nacional De Psiquiatría Ramón de La Fuente, Mexico city, Mexico

Abstract: The endogenous opioid system is associated with a variety of functions, including ethanol reward and reinforcement. Ethanol reinforcement and high alcohol drinking behaviour may be partially mediated by the ethanol-induced activation of endogenous opioid systems. Ethanol and opiates share numerous pharmacological properties and exhibit similar behavioural effects in animals and humans. Low ethanol doses produce psychomotor activation and euphoria, whereas high doses induce locomotor inhibition and sedation. Ethanol may alter opioidergic transmission at different levels, including the synthesis, processing, release and/or ligand binding to opioid receptors. The aim of this work was to investigate the effects of different doses of ethanol on Pro-opiomelanocortin (POMC) mRNA expression in different brain areas. Male Wistar rats were administrated with saline or different doses of ethanol (0.5, 0.75, 1.0, 1.5, 2.0, 3.0 g/kg i.p.) and 30 min later distinct brain areas were dissected: prefrontal cortex (PFC), nucleus accumbens (NAcc), anterior-medial (amCP) and medial-posterior (mpCP) regions of the caudate-putamen (CP), amygdala, hypothalamus and hippocampus. POMC mRNA levels were quantitated by real time PCR. POMC mRNA expression was increased by high ethanol doses in the PFC, while moderate to high doses (1.5 and 2.0 g/kg) decreased POMC mRNA levels in the hypothalamus and hippocampus. Ethanol induced biphasic effects in the NAcc, amygdala and CP. Moderate ethanol doses increased (amCP and mpCP) or decreased (NAcc and amygdala) mRNA expression, while high doses produced the opposite effect in these regions. Our results indicate that ethanol-induced changes in POMC mRNA expression are dose-dependent and region-specific. Ethanol-induced changes in POMC mRNA expression could be involved in both the stimulant and sedative effects of the drug.

Disclosures: K. Hernandez Fonseca: None. **E. Cabrera-Muñoz:** None. **C. Reyes:** None. **M. Mendez:** None.

Poster

257. **Opioids: Neural Mechanisms of Addiction**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 257.19/LL10

Topic: C.18. Drugs of Abuse and Addiction

Support: Department of Veterans Affairs

Department of Defense

Office of Naval Research

Title: Confocal microscopy reveals shrinkage of dopaminergic cell bodies in the ventral tegmental area after access to morphine, but not endomorphin analogs

Authors: *M. R. NILGES[1], J. E. ZADINA[1,2,3];
[1]Grad. Neurosci. Program, [2]Dept of Med. and Pharm, Tulane Univ. Sch. of Med., New Orleans, LA; [3]SE LA Veterans Hlth. Care Syst., New Orleans, LA

Abstract: We have previously demonstrated that endomorphin (EM)-derived analogs are mu-opioid receptor agonists that inhibit pain behaviors with fewer side effects than morphine. For example, side effects from morphine include respiratory depression, tolerance, motor impairment, glial activation, and reward potential. By comparison to morphine and appropriate controls, we have shown that all 5 of these side effects are substantially reduced in rats injected with EM analogs. Abuse of morphine and heroin is pervasive and has a major socio-economic impact. By comparing equi-antinociceptive doses of morphine and EM analogs, we have shown that EM analogs do not elicit significant reward potential in the conditioned place preference model and have reduced self-administration (SA) rates under 12 hour/day/7 day sessions. The rewarding effects of morphine are thought to occur via disinhibition of dopaminergic cells located in the ventral tegmental area (VTA). After chronic morphine usage, the surface area of dopamine (DA) cell bodies in the posterior VTA decrease and may contribute to the reward tolerance associated with morphine. The decreased soma size in the VTA is interesting because it reliably occurs in rodent models of morphine or heroin reward and in post-mortem brains from heroin-dependent humans. In this study, we have directly compared tyrosine hydroxylase-containing DA neurons in the VTA of rats that previously self-administered vehicle, morphine, or EM analogs for 12 hours/day/7 days. Using immunohistochemical techniques and a Leica

confocal microscope with resident software for determining soma surface area, we compared DA soma sizes from the VTA of these rats. Consistent with previous reports, VTA soma sizes were significantly reduced in rats that self-administered morphine compared to controls. However, the surface area of DA neurons in the VTA from rats with access to EM analogs was not different from controls. Together with our previous conditioned place preference and self-administration data, the results provide evidence that 1) EM analogs produce significantly less reward behavior than morphine under identical conditions, and 2) VTA dopamine morphology is not altered by access to EM analogs in a paradigm where morphine is self-administered and reduces DA soma size in the VTA. The results support our hypothesis that the analogs have better safety profiles than morphine.

Disclosures: M.R. Nilges: None. **J.E. Zadina:** None.

Poster

257. **Opioids: Neural Mechanisms of Addiction**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 257.20/LL11

Topic: C.18. Drugs of Abuse and Addiction

Support: NIH Grant DA15446

Title: Impairments of chromatin remodeling and gene expression in the striatum of human heroin abusers

Authors: *G. EGERVARI[1], E. KELLER[2], Y. L. HURD[1];
[1]Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Forensic and Insurance Med., Semmelweis Univ., Budapest, Hungary

Abstract: Heroin abuse continues to have a detrimental impact on the individual and society in the United States and worldwide. Knowledge is, however, still lacking regarding neurobiological disturbances in human heroin abusers that could better guide treatment interventions. Abnormal gene transcription has been noted in opiate users, but no information currently exists regarding related epigenetic mechanisms, key regulators of transcription, in human drug users. As such, we studied the human striatum to characterize the state of epigenetic marks and genes related to synaptic plasticity, dysregulation of which is a core feature of addiction disorders.
We used a homogeneous postmortem collection of human heroin abusers to explore expression of genes in the striatum (microarray, Nanostring, quantitative real-time polymerase chain reaction) directly related to synaptic plasticity and glutamatergic neurotransmission, and to assess associated epigenetic mechanisms (Western blot, chromatin immunoprecipitation).

We observed marked perturbations of glutamatergic gene expression and epigenetic regulation in the striatum of human heroin abusers. In the nucleus accumbens, we found heroin-related transcriptional changes of chromatin remodeling enzymes and of genes involved in synaptic plasticity and glutamatergic neurotransmission. In the dorsal striatum, we found a significant increase in nuclear coactivator 1 (NCOA1) histone acetyltransferase and global histone H3 acetylation (AcH3) that correlated with years of heroin use, and showed negative correlations with heroin toxicology. In addition, we also observed a significant increase in AcH3 at the gene body and promoter region of selected glutamatergic genes. Other marks examined such as tri- or dimethylation of histone H3 lysine-9 (H3K9me3/2) related to transcriptional repression were not significantly altered.
Overall, the data to date suggest that epigenetic perturbations, particularly the hyperacetylation of histone H3, and thus the resulting more open chromatin configuration, might be intimately involved in the regulation of heroin-induced striatal synaptic plasticity. In addition, our findings indicate that molecular mechanisms are differentially affected by acute drug toxicity versus the chronic pathologic state of substance abuse.

Disclosures: G. Egervari: None. **E. Keller:** None. **Y.L. Hurd:** None.

Poster

257. **Opioids: Neural Mechanisms of Addiction**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 257.21/LL12

Topic: C.18. Drugs of Abuse and Addiction

Title: The effects of repeated morphine exposure on metabotropic glutamate receptor activity in adolescent mice

Authors: K. E. SELOFF, M. A. EMERY, M. S. L. BATES, P. J. WELLMAN, *S. EITAN; Dept of Psychology, Behavioral & Cell. Neurosci Program, Texas A&M Univ., College Station, TX

Abstract: Adolescent mice exhibit marked changes in D2/D3 dopamine receptor activity following administration of certain opioids. Thus, the present study examined whether repeated exposure to morphine would also modulate the activity of metabotropic glutamate receptors (mGluRs) in the dorsal striatum of adolescents. Administration of a group I-specific mGluR agonist, S-3,5-dihydroxyphenylglycine (DHPG), induces face-washing behavior in adolescent mice. Since this behavioral effect has been shown to be mediated by mGluR1, this behavior was used as an indicator of mGluR1 activity in the dorsal striatum.

Adolescent mice were first examined for their behavioral response to unilateral administration of DHPG directly into the dorsal striatum. Morphine (20 mg/kg, s.c.) was then administered once daily for 6 days. The response to DHPG was re-examined at one of three times, i.e., 2, 4, or 24 hours following administration of the final dose of morphine.
A significant increase in face-washing behavior was observed in drug-naïve animals following microinjection of a low dose (10 nmol) of DHPG into the dorsal striatum. Importantly, no differences were observed between the experimental groups, either at baseline or post-DHPG. Thus, differences between the various groups observed following repeated morphine administration are not due to pre-existing differences prior to the morphine administration. Morphine administration did not alter baseline face-washing behaviors in any of the experimental groups. Additionally, there were no significant differences in baseline face-washing behaviors between the various experimental groups following the repeated morphine administration. Thus, morphine per se and the withdrawal from morphine have no direct effects on face-washing behavior. Accordingly, differences in DHPG-induced face-washing behaviors between post- and pre-morphine administration are likely due to the effects of morphine administration on mGluR1 activity rather than locomotor activity or stereotyped behaviors in general.
Notably, this study demonstrated that repeated exposure to morphine does have an effect on the DHPG-induced increase in face-washing behaviors. Our results suggest that repeated morphine administration causes a decrease in mGluR1 activity in the dorsal striatum of adolescent mice, which could affect long-term neural activity. This is significant because it contributes to a more complete understanding of the factors that contribute to development of opioid addiction during adolescence.

Disclosures: K.E. Seloff: None. **M.A. Emery:** None. **M.S.L. Bates:** None. **P.J. Wellman:** A. Employment/Salary (full or part-time):; Texas A&M Univ. **S. Eitan:** A. Employment/Salary (full or part-time):; Texas A&M Univ.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.01/MM1

Topic: C.20. Drug Discovery and Development

Support: UDA-POIG.01.03.010-12-100/08-00 co-financed by European Union from the European Fund of Regional Development (EFRD)

Title: IP271973 a novel nonselective mglur7 agonist

Authors: *A. PILC, P. P. BRAńSKI, A. BOJARSKI, A. STANKIEWICZ, G. BURNAT, B. CHRUśCICKA;
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Abstract: **Background:** Available data indicate on the important of mGluR7 receptor in psychiatric disorders. However, the results are misleading. The only known selective agonist for $mGluR_7$, N,N'-dibenzhydryl-ethane-1,2-diamine dihydrochloride (AMN082) was active in preclinical test of depression and anxiety. Concomitantly similar properties were observed for negative allosteric modulator of $mGluR_7$ (MMPIP). This discrepancy may be a result of a rapid internalization of mGluR7 in the presence of agonist and may result an in inhibition of signaling via mGlu7 receptor, as a net effect. Therefore, we need novel agents with
agonistic properties for mGlu7 receptor to evaluate if the activation of the receptor would produce antidepressant- and anxiolytic-like activity. **Aim:** Identification novel chemical scaffold possessing $mGlu_7$ positive allosteric modulation activity by interaction with transmembrane region of $mGlu_7$ receptor. **Methods:** Human GRM7 was cloned into genome of HEK-293 cells contained T-Rex expression system
(Invitrogen). Expression of the receptor was analyzed by means of qRT-PCR and Western blot in both: cells before and after stable transfection with GRM7. The screening study and activity of potential PAM was determined using forskolin-induced cAMP accumulation, in a HEK-293 T-Rex cell line stably expressing $mGlu_7$ or mock transfected HEK-293 T-Rex cell line. For the functional characterization of $mGlu_7$
we measured level of cyclic AMP (cAMP) by cAMP dynamic2 (CisBio) kit according to manufacture instructions. The concentration response curves were fitted using the non-linear regression analysis program, GraphPad Prism (GraphPad Software). All liquid handling operations were performed using EVO 2000 system (Tecan). **Results:** Via *in vitro* screening of compound collection we have identified
chemical scaffold possessing $mGluR_7$ potential PAM activity. Active compound have been characterized: induce a leftward-shift of the glutamate concentration-response curve (8,6 fold in 0,3 uM concentration of IP271973). - Activate $mGluR_7$ as a partial agonist (EC_{50} = 0,3 uM) - Is not selective, activate both $mGluR_4$ and $mGluR_8$

Disclosures: A. Pilc: None. **P.P. Brański:** None. **A. Bojarski:** None. **A. Stankiewicz:** None. **G. Burnat:** None. **B. Chruścicka:** None.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.02/MM2

Topic: C.20. Drug Discovery and Development

Support: the Establishment and Operation of Korea Brain Research Institute (KBRI) Basic Research Program of the Ministry of Science, Ict & future Planning (2031-415)

Title: Dehydroevodiamine·HCl improves stress induced memory impairments and depression like behavior in rats

Authors: *H. KIM[1,2], K. SHIN[1], K.-A. CHANG[3], H. CHOI[2], Y.-H. SUH[2];
[1]Dept. of Pharmacol., Seoul Natl. University/ Col. of Med., Seoul /jongno-Gu, Korea, Republic of; [2]Convergence Brain Res. Dept., Korea Brain Res. Institute(KBRI), Deagu, Korea, Republic of; [3]Gachon Univ., Incheon, Korea, Republic of

Abstract: Dehydroevodiamine·HCl(DHED) has been reported to prevent memory impairment and neuronal cell loss in a rat model with cognitive disturbance. We investigated the effect of DHED on memory impairment and behavioral abnormality caused by stress. We demonstrated that DHED can improve stress-induced memory impairments and depression-like behaviors by using open-field test, Y-maze test and forced swimming test. DHED treatment significantly recovered the decreases in the levels of neural cell adhesion molecule (NCAM) proteins caused by stress and the decreases in cell viability and NCAM levels by staurosporine treatment. Our results suggested that DHED is a potential drug candidate for neuronal death, memory impairment and depression induced by stress.

Disclosures: H. Kim: None. **K. Shin:** None. **K. Chang:** None. **H. Choi:** None. **Y. Suh:** None.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.03/MM3

Topic: C.20. Drug Discovery and Development

Title: Triple tracer receptor occupancy for 5-HT_{1A}, 5-HT_{2A} and D_2 using LC-MS/MS based method in rats

Authors: *G. BHYRAPUNENI, V. KANDIKERE, J. THENTU, D. AJJALA, R. ALETI, R. NIROGI;
Discovery Res., Suven Life Sci., Hyderabad, India

Abstract: Measuring the extent to which the test compound occupies target receptor plays a critical role in prioritizing compounds in CNS drug discovery. Thus, measuring in-vivo

occupancy simultaneously for compounds having affinity towards multiple receptors will be of great interest. Utility of multiple non-radiolabelled tracers to measure their distribution for occupancy at various receptors was accomplished in a single animal using LC-MS/MS mode of analysis. Current research on novel antipsychotics focus interactions with range of receptors, including serotonin and dopamine receptors, the mechanism of action different from typical and atypical antipsychotics. In this report, we developed simultaneous in-vivo receptor occupancy assay for 5-HT_{1A}, 5-HT_{2A} and D_2 using three different non-radiolabelled tracers, WAY 100635, MDL 100,907 and raclopride, respectively at 3 µg/kg, i.v. dose. The tracers used in this study were chosen based on information from the literature describing their use as occupancy tracers. Individual assays for each target were set and then a "triple tracer" assay was established where all three tracers were administered simultaneously. Assay was validated with various doses of selective and non-selective antipsychotics (pindolol 0.1 - 10 mg/kg, i.v, ziprasidone 0.01 - 6.0 mg/kg, i.v or olanzapine 0.1 to 10 mg/kg, p.o) in rats. The ED_{50} vales were similar when tested alone or in triple tracer assay. This is the first reported method to simultaneous determine the occupancy of these receptors in the same animal. The method can be employed in screening the novel compounds at lead optimization phase.

Disclosures: G. Bhyrapuneni: None. **V. Kandikere:** None. **J. Thentu:** None. **D. Ajjala:** None. **R. Aleti:** None. **R. Nirogi:** None.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.04/MM4

Topic: C.20. Drug Discovery and Development

Title: Amygdala-specific phosphodiesterase 2 knockout controls hippocampal remodeling and fear memory

Authors: *Y. XU, C. ZHANG, L. RUAN, T. SHAO, H. ZHANG, J. O'DONNELL;
Dept. of Behavioral Med. and Psychiatry, West Virginia Univ., Morgantown, WV

Abstract: Impaired regulation of emotional memory is a feature of stress-related disorders, such as anxiety and fear memory impairment. Such regulation occurs, in part, by interactions between two stress-sensitive brain regions, the basolateral complex of the amygdala (BLA) and its downstream target the hippocampus. Extensive findings indicate that inactivation of the ipsilateral, but not the contralateral, BLA blocks the expression of genes involved in hippocampal synaptogenesis and hippocampal responses to stress, further indicating an

interaction between the BLA and hippocampal function. However, the molecular and cellular mechanisms supporting the influence of the amygdala on fear memory and hippocampal synaptic plasticity remain unknown. The high expression of phosphodiesterase 2 (PDE2) in the hippocampus and amygdala makes it an attractive target for regulation stress-related emotional memory. Activation of the secondary messengers (cAMP and cGMP) by inhibition of PDE2 appears to be a viable and tractable means of enhancing neuronal communication between these two brain regions. The current PDE2 inhibitor Bay 60-7550, though very potent, is not completely selective over other PDEs. Efforts have been made for the creation of a complete PDE2 knockout mouse, which only led to embryonic lethal pups. Creating conditional PDE2 knockout mice of the brain will provide great research merit in explaining the role of PDE2 in remodeling of impaired neuronal circuits induced by stress. In this study, an amygdala-specific PDE2 knockout mouse will be created to elucidate the involvement of PDE2 in chronic stress, and its function in controlling the remodeling responses of neuronal connections in the hippocampus to emotional input from the ipsilateral BLA. Successful completion of this study will reveal the role of PDE2 in shaping emotion-associated neuronal networks during stress. Characterizing the role PDE2 plays in amygdala-hippocampal associated fear-memory performance could result in a breakthrough in the PDE research area, and possibly identify PDE2 as a potential target for the treatment of stress-related neuropsychiatric disorders.

Disclosures: Y. Xu: None. **C. Zhang:** None. **L. Ruan:** None. **T. Shao:** None. **H. Zhang:** None. **J. O'Donnell:** None.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.05/MM5

Topic: C.20. Drug Discovery and Development

Title: Translational drug discovery at the University of Sussex

Authors: *J. R. ATACK, S. E. WARD;
Translational Drug Discovery Group, Univ. of Sussex, Sch. of Life Sci., Brighton, United Kingdom

Abstract: Big Pharma companies (for example GSK, Astra-Zeneca, Sanofi, Novartis, Merck) are decreasing their drug discovery efforts within neuroscience, especially for psychiatric disorders (ECNP Summit, Eur. Neuropsychopharmacol., 2011, 21:495-9). However, there is little doubt that there remains a need for improved treatments for a variety of CNS disorders,

which raises the question of from where will such new therapies emerge (Schoepp, 2011, Nat. Rev. Drug Discov., 10:715-6)? The onus therefore falls upon academic drug discovery which takes advantage of the fact that academic groups with a deep knowledge of the pathophysiology of disease processes are best-placed to identify novel targets or understand existing targets. Accordingly, the University of Sussex (UoS) recently set up the Translational Drug Discovery Group (TDDG). Our aim is to establish a portfolio of targets within the oncology and neuroscience therapeutic areas that will attract external funding (Wellcome Trust, MRC, CRUK, Pharma or whoever), with the ultimate goal being to provide preclinical candidate molecules suitable for clinical development.
The TDDG comprises a core group of medicinal chemists and biologists that are supplemented by personnel funded by external funders to deliver on specific projects. We can provide our research partners with pharmacological tools to better validate novel targets as well as identify and optimise screening hits against the target of interest. Rather than high-throughput screening, the TDDG has chosen an alternative approach to hit-finding, namely fragment-based drug discovery. Following this strategy, a relatively small number (c.2,000) of low molecular "fragments" are screened using either biochemical or biophysical assays with hits being confirmed using X-ray crystallography, thereby exploiting the UoS's world-leading structural biology expertise. Further limited exploration of novel chemotypes is used to develop an early understanding of the structure-activity relationship in order to support applications to external funding bodies. Our oncology targets are based around UoS expertise in DNA damage whereas the neuroscience portfolio is currently being assembled and we welcome the opportunity to collaborate with any interested academic or industrial partners.

Disclosures: J.R. Atack: None. **S.E. Ward:** None.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.06/MM6

Topic: C.20. Drug Discovery and Development

Support: Taisho pharmaceutical., Co., LTD

Scientific Research (B) from the Japanese Society for the Promotion of Science (JSPS)

Title: Centrally acting antitussives, possessing inhibitory action on GIRK channels, increase the c-Fos and TH double-immunopositive cells in the ventral tegmental area and locus coeruleus

Authors: *R. HAMASAKI[1], F. SOEDA[1], K. TAKAHAMA[1,2];
[1]Dept. of Envrn. and Mol. Hlth. Sci., Grad. Sch. of Pharmaceut. Sci., Kumamoto, Japan;
[2]Kumamoto Hlth. Sci. Univ., Kumamoto, Japan

Abstract: We previously reported that the centrally acting antitussives inhibited inwardly rectifying K^+ (GIRK) channel currents to induce neuronal firing of the neurons in the dorsal raphe, ventral tegmental area (VTA) and locus coeruleus (LC) in rats. Furthermore, we reported that the antitussives at antitussive-effective doses elevated extracellular monoamine levels in rat brain and ameliorated symptoms of the animal models of various psychiatric disorders including intractable depression without causing undesired effects like psychostimulants. According to our previous behavio-pharmacological studies, the multiple pharmacological effects of the antitussives may be produced at least partly by activating catecholaminergic neurons via inhibition of GIRK channels. Then it is essential for validation of our own working hypothesis to confirm whether the antitussives at antitussive-effective doses activate catecholaminergic neurons in vivo, because it is reported that dopaminergic neurons in VTA may be not homogeneous in neurochemical properties and pharmaco-physiological roles. In this study, using double-immunohistochemical technique, we investigated the effects of tipepidine (TP) and cloperastine (CP) on the number of double-immunopositive cells for c-Fos and tyrosine hydroxylase (TH) in VTA and LC of rats minutely. TP and CP (40 mg/kg, i.p.) commonly increased the number of TH(+)/c-Fos(+) cells in the LC(Bregma -6.84 mm), and in the restricted region of VTA (Bregma -5.88 mm), but not in other regions of the VTA (Bregma -6.24 mm and -6.84 mm). Then CP but not TP increased TH(+)/c-Fos(+) cells in the VTA (Bregma -5.16 mm). Furthermore, CP and TP also increased TH(-)/c-Fos(+) neurons in the VTA (Bregma -5.88 mm, -6.24mm, -6.84 mm). Then, CP but not TP increased TH(-)/c-Fos(+) cells in the VTA (Bregma -5.16 mm). Subnuclei (paranigral nucleus, PN; parabrachial pigmented nucleus, PBP; parainterfascicular nucleus, PIF; interfascicular nucleus, IF) analysis of the VTA (Bregma -5.88 mm) is now in progress. The increment of TH(-)/c-Fos(+) cells by TP and CP might suggest that TP and CP also activate GABAergic interneurons, because it is greatly known that there are numerous GABAergic interneurons expressing GIRK channels in the VTA, which may put a brake on excessive excitation of dopaminergic cells. Although further studies are required, the current data suggest that the antitussives possessing inhibitory action on GIRK channel currents, may activate noradrenergic neurons in LC, and dopaminergic neurons of the restricted region in VTA, which may contribute to the multiple pharmacological effects in experimental animals.

Disclosures: R. Hamasaki: 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.; Taisho pharmaceutical Co., LTD. **F. Soeda:** 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.; Taisho pharmaceutical Co., LTD. **K. Takahama:** 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.; Taisho pharmaceutical., Co., LTD.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.07/MM7

Topic: C.20. Drug Discovery and Development

Title: Evaluation of novel drug targets for schizophrenia treatment using a model of cortical and basal ganglia circuitry

Authors: *P. D. ROBERTS[1], H. GEERTS[2], A. SPIROS[1];
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Abstract: Novel targets for treatments of psychiatric diseases may lead to unexpected outcomes such as underestimated efficacy in humans and adverse side effects. Although animal models may reveal some aspects of clinical outcome, computational models of brain circuitry can integrate disparate data to augment predictions of potential clinical success. We present a computational model that is calibrated for improving the positive and negative symptoms of schizophrenia (PANSS) scale and extrapyramidal symptoms (EPS) to demonstrate an evaluation of phosphodiesterase-10 inhibitor (PDE10-I) as a treatment for schizophrenia.
We have developed a biophysical model of the cortex, thalamus and basal ganglia loop to simulate the neural activity patterns associated with healthy and pathological conditions in the human brain. The schizophrenia pathologies are implemented by altering dopamine levels, NMDA and GABA currents, and background noise. Modulatory effects of dopamine, serotonin, and other modulators are incorporated throughout the circuitry by implementing the changes in membrane and synaptic currents caused by receptor activation in each neuron type. Model readouts include firing rates, frequency band power, and information measures of the circuit activity.
The model was calibrated to represent the effects of drugs in human patients by adjusting modulator parameters to optimize the correlation between model readouts and clinical data. We calibrated the model to predict the efficacy of 72 antipsychotics-dose combinations on the PANSS clinical scale and the liability for EPS using historical clinical trials.
We then implemented the effects of PDE10 inhibition in the medium spiny neurons of the striatum by simulating the intracellular signaling pathway effects on dopamine receptor activation mediated modulation of membrane currents and downstream activity changes in the

cortico-striatal-thalamic loop. The simulated results demonstrate the unexpected dystonia motor effects and lack of efficacy as observed in recent clinical trials, while opposite effects are obtained with the rodents. Combination therapy with appropriate doses of dopamine antagonists is shown to improve the clinical profile of PDE10-I if the correct balance between D1- and D2-type receptors is obtained.

Disclosures: P.D. Roberts: A. Employment/Salary (full or part-time):; In Silico Biosciences, Inc. **H. Geerts:** A. Employment/Salary (full or part-time):; In Silico Biosciences, Inc. **A. Spiros:** A. Employment/Salary (full or part-time):; In Silico Biosciences, Inc.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.08/MM8

Topic: C.20. Drug Discovery and Development

Support: Supported in part by SDSU-JRF and SDSU-COP grants

Title: Nicotinic receptor ligand reduces anxiety and depression-like behaviors after withdrawal from voluntary ethanol drinking in mice

Authors: *M. A. RONI, S. RAHMAN;
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Abstract: We have shown that brain nicotinic acetylcholine receptor (nAChR) ligand lobeline decreases anxiety and depression like-behaviors after withdrawal from nicotine self-administration. The present study has determined the effects of lobeline on anxiety and depression-like behaviors after withdrawal from voluntary ethanol drinking. C57BL/6J male mice were allowed to drink 10% ethanol for four weeks. Mice were tested after 24 h and 14 days of ethanol withdrawal. In forced swim test (FST), a measure for depression-like behavior, acute lobeline treatment (1 mg/kg, s.c.) significantly reduced immobility time compared to control after 24 h of withdrawal (control: 124 ± 14 sec, lobeline: 69 ± 5 sec; $p < 0.05$). In elevated plus-maze (EPM), a measure for anxiety-like behavior, lobeline significantly increased open arm entries (control: 11 ± 3%, lobeline: 34 ± 4%; $p < 0.01$) after 24 h of withdrawal. Likewise, after 14 days of ethanol withdrawal, acute lobeline treatment significantly reduced immobility time in FST (control: 108 ± 8 sec, lobeline: 49 ± 12 sec; $p < 0.01$); increased open arm entries (control: 5 ± 2%, lobeline: 20 ± 2%; $p < 0.01$) and open arm times (control: 0.5 ± 0.2%, lobeline: 3 ± 1%; $p < 0.05$) in EPM. Furthermore, the effects of chronic lobeline on anxiety and depression-like behaviors after withdrawal from voluntary ethanol drinking and associated neuroadaptive

changes are being investigated. Overall, these results suggest that lobeline reduces anxiety and depression-like behaviors in mice likely by targeting brain nAChRs and nicotinic cholinergic mechanisms may play a critical role during withdrawal from ethanol dependence.

Disclosures: M.A. Roni: None. **S. Rahman:** None.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.09/MM9

Topic: C.20. Drug Discovery and Development

Title: SUVN-911, α4β2 antagonist shows antidepressant like activity and addresses the limitation of current antidepressant therapy

Authors: *V. BENADE, R. PONNAMANENI, G. BHYRAPUNENI, P. JAYARAJAN, V. GOURA, I. BHATIA, S. TELLA, V. GOYAL, S. PANDEY, S. JANA, A. MOHAMMED, S. VEERAMALLA, R. NIROGI;
Suven Life Sci. Ltd, Hyderabad, India

Abstract: Mental illnesses are leading cause of disability globally. Depression alone affects almost 121 million people worldwide. About one in every 10 persons is expected to present at least one depressive episode during his/her lifetime. Projections for economic expenditure place depression near the top of the list for the year 2030. Currently available antidepressants have a number of limitations. Some of these include low response and remission rates, delayed onset of action, sleep disturbances, cognitive dulling and sexual dysfunction. SUVN-911 is a potent and selective $\alpha_4\beta_2$ antagonist. SUVN-911 demonstrated more than 500 fold selectivity for $\alpha_4\beta_2$ receptors (NovaScreen selectivity panel). SUVN-911 showed good brain penetration and receptor occupancy following oral administration, with good oral bioavailability in all preclinical species. SUVN-911 was evaluated in animal models of depression like the DRL-72s, dominant submissive assay and forced swim assay. The minimum effective dose of SUVN-911 was found to be less than 1 mg/kg, *p.o.* At behaviorally effective doses, SUVN-911 produced a significant increase in cortical serotonin levels. SUVN-911 showed good margin of safety in toxicity studies and is non-mutagenic. The pharmacology, pharmacokinetic, metabolic, biopharmaceutical and toxicity profiles of SUVN-911 makes it promising candidate for clinical development. Above observations provide strong support to develop SUVN-911 for the management of mood disorders. The compound is ready for clinical evaluation.

Disclosures: V. Benade: None. **R. Ponnamaneni:** None. **G. Bhyrapuneni:** None. **P. Jayarajan:** None. **V. Goura:** None. **I. Bhatia:** None. **S. Tella:** None. **V. Goyal:** None. **S. Pandey:** None. **S. Jana:** None. **A. Mohammed:** None. **S. Veeramalla:** None. **R. Nirogi:** None.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.10/MM10

Topic: C.20. Drug Discovery and Development

Title: Scopolamine and ketamine attenuate parachlorophenylalanine-induced behavioral despair in a stress-sensitive rat strain

Authors: *W. A. ECKERT, III, G. CHEN;
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Abstract: Parachlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, interrupts the chain of synthesis of the important neurotransmitter, serotonin. PCPA administered systemically to humans can cause depression relapse in recovered patients under treatment with antidepressants. The relapse lasts for 4 to 7 days after PCPA withdrawal. Previous rodent studies showed that PCPA treatment can reduce serotonin levels in the brain, increase immobility and evoke anhedonia-like behaviors. Here, we conducted a series of experiments to refine and validate the PCPA paradigm. We found that 3 daily doses of PCPA in stress-sensitive Wistar Kyoto (WKY) rats can induce a long-lasting despair-like behavior using the active avoidance test (AAT). PCPA-treated rats failed to escape or avoid escapable foot shock up to 22 days after the last PCPA treatment. By contrast, vehicle-treated rats escaped or avoided shock on each day tested. There was no significant difference between groups in hot plate responses suggesting that the deficits of PCPA-treated rats in the AAT were not likely confounded by an altered pain threshold. Intravenous infusion of the muscarinic receptor antagonist, scopolamine, and the *N*-Methyl-D-aspartic acid (NMDA) antagonist, ketamine, each have been reported to rapidly reduce the severity of depression in patients. Both scopolamine and ketamine significantly attenuated the PCPA-induced increase in escape latencies in WKY rats in the AAT in a temporal manner similar to those observed in depression patients. These data support the administration of PCPA in stress-sensitive WKY rats to produce a model of depression that may be useful for mechanistic research and drug discovery and development.

Disclosures: **W.A. Eckert:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **G. Chen:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C..

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.11/NN1

Topic: C.20. Drug Discovery and Development

Title: Activity of serotonin 5-ht1a receptor biased agonists in rat models of depression and syndrome: Superior profile of the post-synaptic preferential agonist, f15599

Authors: M. J. O'CALLAGHAN[1], W. C. LI[1], *R. A. MCARTHUR[2], M. VARNEY[3], A. NEWMAN-TANCREDI[3];
[1]Cerca Insights, Penang, Malaysia; [2]McArthur & Assoc GmbH, Basel, Switzerland;
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Abstract: BACKGROUND: Serotonin 5-HT1A receptors have been extensively investigated in the mechanism of action of antidepressants. Indeed, 5-HT1A agonists are active in animal models of depression. However, previous drugs targeting 5-HT1A receptors, such as buspirone and its analogues, have yielded disappointing clinical antidepressant efficacy, possibly due to poor receptor selectivity and weak partial agonism. In addition, previous drugs did not distinguish between sub-populations of 5 HT1A receptors in different brain regions. This is important because activation of post-synaptic cortical 5 HT1A receptors is considered pivotal to antidepressant and pro-cognitive activity, whereas desensitization of pre-synaptic inhibitory somatodendritic 5 HT1A autoreceptors is a limiting factor in the onset of therapeutic efficacy. Recently, a highly-selective and efficacious 5-HT1A receptor "biased agonist", F15599, has been described that preferentially activates cortical post-synaptic 5-HT1A receptors. Its congener, F13714, displays the opposite profile, preferentially activating pre-synaptic 5 HT1A receptors. The prototypical 5-HT1A agonist, 8-OH-DPAT activates both pre- and post-synaptic 5-HT1A receptors. Here we compared the activities of these drugs in the rat FST, in tests of "syndrome" (induction of flat body posture, FBP, fore-paw treading, FPT, and lower lip retraction, LLR), and in measures of body temperature.
RESULTS: F15599 and F13714 both potently and completely abolished immobility in the FST (MEDs 0.08 mg/kg IP) as did 8-OHDPAT (MED 0.09). F13714 also elicited LLR (MED 0.16), FBP (0.63) and FPT (2.5) as did 8-OHDPAT - LLR (MED 0.1), FBP (MED 0.09.) and FPT (MED 1.78). In contrast, F15599 elicited these behaviors at higher doses (MEDs: LLR 0.63; FBP

0.63; FPT 10). In addition, F15599 showed little propensity to elicited hypothermia (MED 0.63). CONCLUSIONS: these data show that the post-synaptic preferential “biased agonist” F15599 shows potent antidepressant-like activity with a decreased propensity to elicit syndrome. Similar data have been previously reported by another laboratory [1] and the present results constitute their first independent confirmation. Preferential targeting of post-synaptic 5 HT1A receptors may be promising strategy for improved antidepressant therapy.
[1] Assié et al. Int J Neuropsychopharmacol. 2010 Nov;13(10):1285-98.

Disclosures: M.J. O'Callaghan: 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.; Cerca Insights. **W.C. Li:** A. Employment/Salary (full or part-time):; Cerca Insights. **R.A. McArthur:** F. Consulting Fees (e.g., advisory boards); McArthur and Associates, GmbH. **M. Varney:** A. Employment/Salary (full or part-time):; Neurolixis, Inc. **A. Newman-Tancredi:** A. Employment/Salary (full or part-time):; neurolixis, Inc.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.12/NN2

Topic: C.20. Drug Discovery and Development

Title: Antidepressant and anxiolytic profiles of newly synthesized arginine vasopressin V1b receptor antagonists: TASP0233278 and TASP0390325

Authors: *M. IIJIMA, T. SHIMAZAKI, K. FUKUMOTO, S. CHAKI;
Mol. Function and Pharmacol. Labs., Taisho Pharmaceut. Co., Ltd., Saitama,Saitama, Japan

Abstract: Arginine vasopressin (AVP) has been presumed to be involved in stress-related disorders such as depression and anxiety. We revealed that both TASP0233278 and TASP0390325 are potent, selective and orally effective antagonists for an AVP receptor subtype 1b (V1bR). In the present study, we investigated antidepressant and anxiolytic profiles of TASP0233278 and TASP0390325 in animal models of depression and anxiety. Oral administration of TASP0233278 or TASP0390325 exerted the antidepressant effects in two models of depression (forced swimming test and olfactory bulbectomy model). Moreover, TASP0233278 improved depressive-like behavior induced by repeated treatment with corticosterone, a model which has been shown to disrupt hypothalamic-pituitary-adrenal axis function and to be resistant to treatment with currently prescribed antidepressants. In addition to

depression models, TASP0233278 and TASP0390325 exerted anxiolytic effects in several anxiety models (social interaction, elevated plus-maze, stress-induced hyperthermia, separation-induced ultrasonic vocalization). Of note, TASP0233278 counteracted anxiety-like behavior induced by swim stress in the elevated plus-maze test, consisted with anti-stress effects of V1bR antagonists. Moreover, TASP0233278 counteracted sodium lactate-induced panic-like responses in panic prone rats. The present results provide further evidence that V1bR is related to stress-induced changes in behaviors, and that V1bR antagonists may be useful for the treatment of depression and anxiety disorders.

Disclosures: M. Iijima: None. **T. Shimazaki:** None. **K. Fukumoto:** None. **S. Chaki:** None.

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.13/NN3

Topic: C.20. Drug Discovery and Development

Title: *In vitro* and *Ex vivi* profiles of newly synthesized arginine vasopressin V1b receptor antagonists: TASP0233278 and TASP0390325

Authors: *T. YOSHIMIZU, T. SHIMAZAKI, K. TOKUGAWA, S. CHAKI;
Taisho Pharmaceut. Co., Ltd., Saitama, Japan

Abstract: Abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis activity have been observed in patients with stress-related disorders such as depression and anxiety. Arginine-vasopressin (AVP) has been considered the primary factor in the regulation of HPA axis activity through its receptor subtype, V1b receptor (V1bR) and many studies have shown the potential utility of V1bR antagonists for the treatment of depression and anxiety. Here we show pharmacological profiles of TASP0233278 and TASP0390325, novel and potent non-peptide V1bR antagonists. TASP0233278 and TASP0390325 showed high affinities for V1bR, and exhibited much lower affinities for other receptors and enzymes, including V1aR, V2R, or OXTR. TASP0233278 and TASP0390325 also potently attenuated AVP-induced [Ca2+]i increase in CHO cells expressing V1bR, while they did not change basal [Ca2+]i levels in the cells, indicating that both compounds are antagonists for V1bR. Furthermore, we examined antagonism to dDAVP (a synthetic replacement for AVP)-induced potentiation of the effect of corticotrophin-releasing factor (CRF) on ACTH release in rats. Oral administration of TASP0390325 significantly inhibited the CRF/dDAVP-induced plasma ACTH increase in rats. These results show that both TASP0233278 and TASP0390325 are potent, selective and orally

effective V1bR antagonists, and that these compounds can be used as tools to explore pharmacological significance of blockade of V1bR.

Disclosures: T. Yoshimizu: A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co., Ltd. **T. Shimazaki:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co., Ltd. **K. Tokugawa:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co., Ltd. **S. Chaki:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co., Ltd..

Poster

258. **Affective Disorders and Schizophrenia**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 258.14/NN4

Topic: C.20. Drug Discovery and Development

Title: Behavioural and Hypnotic effect of the methanolic extract of Annona senegalensis is rats

Authors: *O. O. SUNDAY[1], P. AKINGBALA[2], O. D. OKOKO[3];
[1]Dept. Of Pharmacol., Univ. of Jos, Jos, Plateau State, Nigeria; [2]Dept. of Pharmacol., Univ. of Jos, Jos, Plateau, Nigeria; [3]Dept. of Pharmacol., Univ. of Jos, Jos, Plateau State, Nigeria

Abstract: Annona senegalensis is used in combination with other herbs in folkloric medicine for the management of psychosis. The hypnotic, effects on phenobabitone induced sleep and behavioural effect of A. senegalensis were investigated in wistar rats. A. senegalensis did not induced sleep in experimental animals, but it was observed that the methanolic extract sedated and attenuated behavioural activity in rats. The results from the study also revealed that A. senegalensis prolonged phenobarbitone induced sleep. The calming effects observed with A. senegalensis may be the basis for its use with other herbs for the management of psychosis.

Disclosures: O.O. Sunday: A. Employment/Salary (full or part-time):; Department of Pharmacology, University of Jos, Jos, Nigeria.. **P. Akingbala:** None. **O.D. Okoko:** None.

Poster

259. **Striate Cortex: Response Properties I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 259.01/NN5

Topic: D.04. Vision

Support: NIH Grant EY018251

Title: The Tortoise and the Hare: Fast vs. very slow corticogeniculate axons differ in visual response properties

Authors: *C. R. STOELZEL[1], Y. BERESHPOLOVA[1], J. ZHUANG[1], J.-M. ALONSO[1,2], H. A. SWADLOW[1,2];
[1]Dept. of Psychology, Univ. of Connecticut, Storrs, CT; [2]Dept. Biol. Sci., SUNY-Optometry, New York, NY

Abstract: The lateral geniculate nucleus (LGN) projects to both layers 4 and 6 of the visual cortex (V1), with layer 6 providing a feedback projection back to the LGN. This feedback is thought to shape receptive field properties and influence spike timing of thalamic neurons. Layer six corticogeniculate (CG) neurons display a wide range of axonal conduction times (40 ms, Swadlow and Weyand, 1987), and little is known how such variations are related to receptive field tuning and visual responding. To examine this question, we studied the receptive field properties of CG neurons in the awake rabbit, mapping their tuning properties with drifting gratings and other stimuli. CG neurons of layer 6 were identified by antidromic activation. Our preliminary results consist of 25 visually responsive corticogeniculate neurons. All of these cells had "simple" receptive fields and had antidromic latencies of 2.5 to 35 ms, median = 18.0 ms). Both fast and slow CG neurons had similarly low rates of spontaneous firing (median = 0.14 spikes/s, range = 0.01 - 2.5). However, fast CG neurons differed in their visual response properties from those with slowly conducting axons in several ways: (1) Fast CG neurons were more responsive (higher F1) during optimal drifting grating stimulation ($r = -.71$, $p < .001$). (2) Fast CG neurons had shorter response latencies to a flash stimulus ($r = .56$, $p<0.01$). (3) Fast CG neurons had shorter interspike intervals during visual stimulation ($r= .69$, $P<0.01$). (4) Fast CG neurons were more sensitive to contrast (C-50) ($r= 0.54$, $p<0.02$), and (5) fast CG neurons preferred higher velocity stimuli ($r= -0.51$, $p<0.02$). Additionally we recorded from 19 corticogeniculate neurons which were identified by antidromic activation, yet exhibited little to no spontaneous activity and were not responsive to visual stimulation by either drifting or flickering grating, more slashing bars of light. These "Silent" cells had longer antidromic latencies (9.4 to 48 ms (mean = 28 ms, t-test $t = 3.34$, $p = 0.002$) than corticogeniculate neurons which responded to visual stimuli. Taken together these results indicate that the diversity in CG axonal delays is strongly related to the diversity in the visual response properties of this system.

Disclosures: C.R. Stoelzel: None. **Y. Bereshpolova:** None. **J. Zhuang:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

259. **Striate Cortex: Response Properties I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 259.02/NN6

Topic: D.04. Vision

Support: EY018251

Title: Layer 4 in primary visual cortex of the awake rabbit: Contrasting properties of simple cells and putative feed-forward inhibitory interneurons

Authors: *J. ZHUANG[1], C. R. STOELZEL[1], Y. BERESHPOLOVA[1], J. M. HUFF[2], X. HEI[1], J.-M. ALONSO[3,1], H. A. SWADLOW[1,3];
[1]Dept. of Psychology, Univ. of Connecticut, Storrs, CT; [2]Ctr. for Neurosci., Univ. of California, Davis, Davis, CA; [3]Dept. of Biol. Sci., SUNY Col. of Optometry, New York, NY

Abstract: Extracellular recordings were obtained from two cell classes in layer 4 of the awake rabbit primary visual cortex (V1): putative inhibitory interneurons (suspected inhibitory interneurons, SINs) and putative excitatory cells with simple receptive fields. SINs were identified solely by their characteristic response to electrical stimulation of the lateral geniculate nucleus (LGN, 3+ spikes at > 600 Hz), and simple cells were identified solely by receptive field structure, requiring spatially separate ON and/or OFF subfields. Notably, no cells met both criteria, and we studied 62 simple cells and 33 SINs. Fourteen cells met neither criterion. These layer four populations were markedly distinct. Thus, SINs were far less linear (F1/F0 < 1), more broadly tuned to stimulus orientation, direction, spatial and temporal frequency, more sensitive to contrast, had much higher spontaneous and stimulus-driven activity, and always had spatially overlapping ON/OFF receptive subfields. SINs responded to drifting gratings with increased firing rates (F0) for all orientations and directions. However, some SINs showed a weaker modulated (F1) response sharply tuned to orientation and/or direction. SINs responded at shorter latencies than simple cells to stationary stimuli, and the responses of both populations could be sustained or transient. Transient simple cells were more sensitive to contrast than sustained simple cells and their visual responses were more frequently suppressed by high contrasts. Finally, cross correlation between LGN and SIN spike trains confirmed a fast and precisely timed monosynaptic connectivity, supporting the notion that SINs are well-suited to provide a fast feed-forward inhibition onto targeted cortical populations.

Disclosures: J. Zhuang: None. **C.R. Stoelzel:** None. **Y. Bereshpolova:** None. **J.M. Huff:** None. **X. Hei:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

259. **Striate Cortex: Response Properties I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 259.03/NN7

Topic: D.04. Vision

Support: NIH Grant EY018251

Title: V1 corticogeniculate input to LGN is sustained

Authors: *Y. I. BERESHPOLOVA[1], C. R. STOELZEL[1], J. ZHUANG[1], J.-M. ALONSO[1,2], H. A. SWADLOW[1,2];
[1]Dept. of Psychology, Univ. of Connecticut, Storrs, CT; [2]Dept. Biol. Sci., SUNY-Optometry, New York, NY

Abstract: Layer 6 of primary visual cortex (V1) receives direct input from the lateral geniculate nucleus (LGN), and is also an important output layer, from which descending corticothalamic projections arise. In the rabbit, LGN concentric neurons are bimodally distributed into sustained and transient populations with distinct response properties (Swadlow and Weyand, 1985; Bezdudnaya et al., 2006; Cano et al., 2006) and each of these cell types provide input to cortical layers 4 and layer 6 (Stoelzel et al., 2008). Examinations of the responses of simple cells and putative inhibitory interneurons (suspected inhibitory interneurons, SINs) in layer 4 to stationary, maintained stimuli have also revealed classes of cells that respond in a sustained or transient manner to maintained stimulation of the receptive field center (Zhuang et al., 2013). Here we extend this analysis to layer 6. Corticogeniculate (CG) neurons were identified by antidromic activation. SINs of layer 6 were identified by their characteristic response to electrical stimulation of the LGN (3+ spikes at > 600 Hz), and had short-duration spikes. As in our previous studies, testing for sustained/transient responding was performed when awake rabbits were in an alert EEG state. We found that visually responsive CG neurons displayed a very broad range of axonal conduction delays (2.5 to 35 ms) and all had simple receptive fields, consisting of one or more spatially separate ON and/or OFF subfield. Remarkably each of the 22 CG neurons that we studied responded in a sustained manner to static stimulation of the receptive field center. This contrasts to our results in layer 4, where simple cells respond in a sustained (43%), transient (26%) or intermediate (31%) manner to maintained stimulation of the RF center (Zhuang et al., 2013). By contrast, SINs of layer 6 were similar to those of layer 4, in yielding predominantly transient responses to static stimulation (6 of 18 layer 6 SINs were sustained, compared to 8 of 34 layer 4 SINs). We also measured the latency of the response to flashing stationary stimuli. CG cells showed significantly longer latencies to visual stimuli (median: 64.6 ms) than layer 6 SINs (median: 33.8 ms; $p<0.001$, K-S test), layer 4 SINs (median: 28.2 ms; $p<0.001$, K-S test) or layer 4 sustained simple cells (median: 37.5 ms, $p< .001$, K-S

test). Thus, our results indicate that the layer 6 feedback to the LGN is overwhelmingly sustained across the broad spectrum of axonal conduction delays seen in this system.

Disclosures: Y.I. Bereshpolova: None. **C.R. Stoelzel:** None. **J. Zhuang:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

259. **Striate Cortex: Response Properties I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 259.04/NN8

Topic: D.04. Vision

Support: P30 NS069339

Title: The rapid emergence of direction selectivity across all layers of ferret primary visual cortex

Authors: *N. RITTER[1], A. ROY[1], J. OSIK[2], S. D. VAN HOOSER[2];
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Abstract: In ferret visual cortex, direction selectivity develops in the days and weeks following eye opening through a process that requires visual experience (Li et al 2006). Direction selectivity can be induced rapidly in visually naïve, isofluorane-anesthetized animals by 3-12 hours of visual stimulation with a moving but not a flashing stimulus (Li et al 2008). This rapid emergence of direction selectivity has been characterized with intrinsic signal imaging and two-photon calcium imaging, techniques that target layer 2/3 neurons and do not give a direct readout of spiking activity.
Naturally emerging direction selectivity has been measured at the level of spiking across all layers (Clemens et al 2013). It was found that layer 4 neurons exhibited direction selectivity earlier in development than layer 2/3 neurons. Additionally, the increase in direction selectivity was initially due to an increased response to motion in the preferred direction, and later increases in selectivity were due to a decreased response to motion in non-preferred directions.
Here we characterize the progression of events during the rapid emergence of direction selectivity that occurs upon exposure to a motion stimulus for several hours. We monitor emerging direction selectivity in all layers with a linear array of electrodes. Data will be presented regarding the direction tuning of V1 neurons from all cortical layers over the course of training. We will compare the progression of cortical tuning during rapid emergence of direction selectivity with the natural progression of cortical tuning in the days after eye opening.

Disclosures: N. Ritter: None. **A. Roy:** None. **J. Osik:** None. **S.D. Van Hooser:** None.

Poster

259. **Striate Cortex: Response Properties I**

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Program#/Poster#: 259.05/NN9

Topic: D.04. Vision

Support: NSF ISO-1120938

MLSC Massachusetts Life Sciences Center grant

Title: Flexibility of cortical tuning during development: Insights from training with unnatural motion stimuli

Authors: *A. ROY[1], J. J. OSIK[2], N. J. RITTER[2], S. VAN HOOSER[2];
[1]Brandeis Biol., Waltham, MA; [2]Biol., Brandeis Univ., Waltham, MA

Abstract: Neurons in ferret V1 not only respond preferentially to bars of specific orientations, but also to one direction of motion orthogonal to the preferred orientation. This direction selectivity (DS) of response, like orientation selectivity (OS), expresses first at the level of cortex and neurons with similar DS are organized into distinct direction columns. But unlike OS, DS develops through normal visual experience during an early critical period (P28-35). In ferrets of this age, neurons in superficial layers of V1 can rapidly gain DS following a few hours of exposure to bidirectionally moving gratings. How does visual experience interact with the genetically specified mechanisms of cortical development to shape DS? There are two fundamentally distinct ways in which the experience-dependent and -independent processes can interact in cortex. According to one view, the genetically specified processes lay out the basic circuit required for DS already before eye opening and subsequent visual experience simply helps the completion of those processes leading to mature DS. In this scenario cortical neurons in P28-35 ferrets should be seeded for their eventual DS. Consistent with this idea, V1 neurons in P28-35 ferrets with limited visual experience exhibit very small biases in direction tuning that strongly predict the tuning outcome following directional training. Alternatively, genetic mechanisms might only establish the OS circuit, leaving visual experience to exert a more instructive role in shaping DS. Consistent with this idea, training with unidirectional grating stimuli shows that the quality of training stimulus also exerts a strong effect on neurons' DS fate. To disentangle the distinct roles played by experience-dependent and -independent processes in DS development, we have developed two types of unnatural motion stimuli that are unlikely to be represented in the form of small biases in the naïve cortex of a normal ferret. One such

stimulus consists of luminance-alternating counter-phase gratings, which is the sum of two gratings moving in opposite directions simultaneously. The second stimulus consists of gratings with arbitrary phase sequences resulting in non-smooth motion. Using in vivo two-photon imaging of calcium signals in P28-35 ferrets we are testing the results of 3-6 hours of training with these stimuli. Data will be presented regarding the directional tuning of layer 2/3 neurons before and after such training. The nature of the selectivity that arises in response to these different training patterns will reveal the degree to which cortex is primed to learn motion selectivity per se versus any arbitrary spatiotemporal pattern.

Disclosures: A. Roy: None. **J.J. Osik:** None. **N.J. Ritter:** None. **S. Van Hooser:** None.

Poster

259. **Striate Cortex: Response Properties I**

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Topic: D.04. Vision

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Columbia University RISE

Gatsby Foundation

DANA Foundation

Fundação para a Ciência e a Tecnologia scholarship SFRH/BD/33276/2007

Title: Reward modulates the task-related hemodynamic signal in primary visual cortex (V1) of alert macaques

Authors: M. M. B. CARDOSO[1,2], M. BEZLEPKINA[1], B. R. LIMA[1], Y. B. SIROTIN[3], *A. DAS[1];
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Abstract: V1 imaging signals recorded from subjects performing visual tasks contain prominent non-visual information such as task timing (Donner et al., 2008; Pestilli et al., 2011). The neural and behavioral correlates of such non-visual signals, and their relation to visually evoked signals,

are poorly understood. By recording simultaneous imaging and electrode signals from V1 in alert monkeys we earlier reported a task-related signal that entrains to task timing and is not predicted by local spiking (Sirotin & Das 2009). When the task is stereotyped this signal can be linearly separated from the stimulus-evoked signal that is, by contrast, strikingly well predicted by local spiking (Cardoso et al., 2012).
Here we report that the task-related signal is modulated by reward (juice) amount, but in a manner that remains poorly related to local spiking. The experiments were structured as in our earlier work (Sirotin & Das, 2009). We recorded electrode signals simultaneously with intrinsic-signal optical imaging (local blood volume) signals from V1 of rhesus macaques performing a periodic fixation task in essentially complete darkness. Here, however, we systematically varied the reward schedule, with the animal receiving alternating blocks of high and low reward for correct trials.
Alternating the reward size led to a consistent change in the task-related signal, with higher peak-to-peak signal amplitude but asymptotically lower trial-averaged mean blood volume for the blocks of high vs. low reward.
Notably, the V1 spiking recorded during dark-room fixation also had a distinct trial-linked fluctuation (which is not due to stray visual input, as established through controls). However, this spiking appears independent of the task-related hemodynamic signal or its changes with reward size. The spiking signal poorly predicted the task-related signal when convolved with visually stimulated hemodynamic response function (HRF) kernels calculated for the same recording site (c.f. Cardoso et al., 2012). HRF kernels obtained by fitting the spikes to the dark-room signal directly gave better predictions; but the kernels were highly variable, both across sites, and for high vs. low reward at the same site, suggesting that they reflect just a fortuitous fit.
To conclude - we show a prominent reward-related modulation of the (hemodynamic) neuroimaging signals recorded from V1 in task-engaged macaques. However, this reward signal appears to be driven by a neural mechanism that remains distinct from mechanisms underlying any local spiking responses. These results will help provide insights for understanding the neural basis of brain imaging.

Disclosures: M.M.B. Cardoso: None. **A. Das:** None. **M. Bezlepkina:** None. **B.R. Lima:** None. **Y.B. Sirotin:** None.

Poster

259. **Striate Cortex: Response Properties I**

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Columbia University RISE

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Fundação para a Ciência e a Tecnologia scholarship SFRH/BD/33276/2007

Title: Spiking activity and not LFP best predicts stimulus-evoked hemodynamics in V1

Authors: *B. R. LIMA[1], M. M. B. CARDOSO[1,2], Y. B. SIROTIN[3], A. DAS[1];
[1]Dept. of Neurosci., Columbia Univ., New York, NY; [2]Champalimaud Fndn., Lisboa, Portugal;
[3]Rockefeller Univ., New York, NY

Abstract: Neuroimaging techniques based on measuring hemodynamics, such as Functional Magnetic Resonance Imaging (fMRI) are widely used for studying human brain activity. Our lab has shown that the hemodynamic signal measured in V1 of alert monkeys performing periodic tasks can be linearly separated into two components: 1- a task-related part that entrains to trial onset and is not predicted by local neural activity and, 2- a stimulus-evoked part which can be well predicted by local spiking activity. In this study we systematically investigate which neural signal (i.e. spiking, vs. different components of local field potential - LFP) best predicts stimulus-evoked hemodynamics.

We performed simultaneous intrinsic-signal optical imaging (a high-resolution, optical analog of fMRI) and electrode recordings in V1 of alert macaque monkeys engaged in passive fixation tasks (N = 91 sites, 5 hemispheres in 3 monkeys). Hemodynamics, LFP and MUA (multi-unit spiking activity) were simultaneously acquired while presenting gratings of a full range of contrasts (typically 6.25-100% in 2x steps, and a blank). The LFP spectrograms were separated into frequency bands comprising 4-12 Hz, 12-30 Hz, 30-90 Hz or 110-200 Hz which our data showed as having distinct functional relationships with the imaging signal. Stimulus-evoked responses were obtained by subtracting away the blank (0% contrast) response from each signal. Response linearity between aggregate neural and hemodynamic signals was estimated by linear regression as well as by fitting the signals using a gamma-variate hemodynamic response function.

Spiking activity was the neural signal that best correlated with stimulus-evoked hemodynamics. LFP power at frequencies greater than 100 Hz was the second best neural correlate, possibly due to spectral leakage of spiking into high LFP frequencies. Power at the gamma band (30-90 Hz) was characterized by weak induction at low contrasts, and strong induction at intermediate and high contrast stimulation compared to spiking or hemodynamics. This resulted in a non-linear relationship with hemodynamics making it a consistently weaker predictor compared to spiking.

Power at low LFP frequency bands (4-12 Hz) was inversely related to hemodynamics for a subset of the recorded sites. However, population results for the low frequencies, despite showing the same trend, were highly variable. The beta frequency band (12-30 Hz) behaved in a manner similar to the gamma band, but its correlation with hemodynamics was consistently weaker. Evoked LFP trace, as well as its square (net LFP power), correlated well with hemodynamics for a few sites but population results revealed them as poor predictors.

Disclosures: B.R. Lima: None. **M.M.B. Cardoso:** None. **Y.B. Sirotin:** None. **A. Das:** None.

Poster

259. **Striate Cortex: Response Properties I**

Location: Halls B-H

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Program#/Poster#: 259.08/NN12-DP7

Topic: D.04. Vision

Title: A relativistically-invariant gabor-like filter for receptive field representation in the mammalian visual cortex

Authors: *S. G. ODAIBO[1,2];
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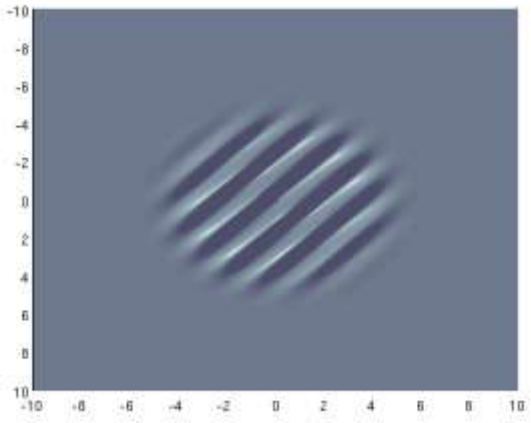
Abstract: The retina is the functional interface between light from a visual scene and the brain's visual cortex, where spatiotemporal representation and computing of visual information begin. Relativistic effects are therefore likely to play a significant role in the retina and retino-cortical axis. However, existing filters used in computational models of visual perception are not relativistically-invariant. In this paper, we introduce a relativistically-invariant 3D spatiotemporal Gabor-like filter, for representing the receptive fields of simple neuronal cells. We will call it the Gabor-Einstein filter. Computational simulations are presented demonstrating its behavioral adherence to pertinent physiological constraints.



Disclosures: S.G. Odaibo: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Chief Scientist and Founder of Quantum Lucid Research Laboratories, An Optical Biotechnology Company.

Poster

259. **Striate Cortex: Response Properties I**

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Topic: D.04. Vision

Support: Grant-in-Aid for JSPS Fellows

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Title: Functional role of acetylcholine in the primary visual cortex of rat

Authors: *S. SOMA[1,2], S. SHIMEGI[1,2], N. SUEMATSU[1], R. MIZUYAMA[3], H. SATO[1,2]; [1]Grad. Sch. of Frontier Biosci., [2]Grad. Sch. of Med., [3]Departments of Biol. Sci., Osaka Univ., Osaka, Japan

Abstract: Acetylcholine (ACh) is known to modulate neuronal activity in the rodent primary visual cortex (V1). We recently examined effects of a microionophoretically administered ACh in V1 of anesthetized rats, finding that ACh facilitated or suppressed visual responses to varying stimulus contrasts by multiplying the control responses, i.e. response gain control. This result raises important questions: 1) why opposing effects are concomitantly observed in a cortical area, 2) how interlaminar circuitry is modulated by ACh, and 3) whether ACh improves the detectability of visual contrast. To examine these points, we combined extracellular multi-unit

recordings and topical administration of ACh, and measured V1 neuronal responses to drifting sinusoidal grating stimuli in anesthetized rats. We confirmed that ACh changes the response gain upward or downward in facilitated or suppressed cells, respectively. These ACh effects showed a laminar bias, where the response suppression and facilitation prevailed in layers 2/3 and layer 5, respectively. Next, we examined ACh effects on the signal-to-noise (S/N) ratio and the grating-phase information calculated as F1/F0 ratio. In facilitated cells, ACh improved the S/N ratio, while in suppressed cells it enhanced the F1/F0 ratio without any concurrent reduction in the S/N ratio. These effects on S/N and F1/F0 ratios were observed in regular-spiking cells, but not in fast-spiking cells. Our findings suggest that ACh promotes the signaling of grating-phase information from supragranular cells to higher-order areas by the suppressive modulation, and enhances feedback signals with a high S/N ratio from infragranular cells to subcortical areas by the facilitatory modulation. To examine whether such fine regulation of visual information processing by ACh contributes to the improvement of visual performance in behaving animals, we trained rats to detect visual stimulus in a two-alternative forced-choice task combined with a staircase method and found that donepezil, a cholinesterase inhibitor, improved the contrast sensitivity depending on the stimulus conditions.

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Poster

259. **Striate Cortex: Response Properties I**

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Topic: D.04. Vision

Support: NSFC grant 31125014

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NIH grant EY007968

Title: Dual labeling of figural elements in primary visual cortex

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Abstract: According to the "labeled line" theory in sensory processing, different neurons in the primary visual cortex (V1) register different local stimulus features; on the other hand, V1 neuronal responses can be affected by global stimulus context. Contextual influences in V1 have been interpreted to play diverse roles in mediating various center-surround interactions, but it is unclear whether the V1 signals conveying figural information defined by different stimulus contexts share similar properties. By examining the response properties of V1 neurons in monkeys detecting different forms of targets within complex backgrounds, we found that V1 plays a double role in stimulus labeling: registering the local stimulus attributes and marking the local elements within the global context as belonging either to the figure or to the background. The latter global labeling, which is superimposed on but can be dissociated from the local labeling, is independent of neuronal feature selectivities and of the global figural attributes when the saliency of the figure is fixed. For instance, when the figure to be detected was a global contour made of collinear lines embedded in randomly oriented lines, we could dissociate a general additive response component that was dependent on the global saliency of the contour but was independent of neuronal orientation selectivity and of the global contour orientation. Moreover, this additive effect was indistinguishable when the neuron's receptive field was centered on any local element lying on the contour path, even if the element in the receptive field was in marked feature contrast with the other stimulus elements. However, the response component associated with the global labeling gradually faded when the luminance contrast between the background and the figure was increased, indicating dependence of the global labeling process on the complex background. The other forms of stimuli with global figure-ground configurations showed similar results, suggesting a general mechanism for figure-ground processing in V1.

Disclosures: M. Chen: None. **C. Gilbert:** None. **W. Li:** None.

Poster

259. **Striate Cortex: Response Properties I**

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Topic: D.04. Vision

Support: National, SNSF: ProDoc PDFMP3_127179

Title: Infrequent simple cells in the tree shrew primary visual cortex

Authors: *J. VEIT, A. BHATTACHARYYA, R. KRETZ, G. RAINER;
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Abstract: There is a notable difference in the occurrence of primary visual cortex (V1) „simple“ and „complex“ cells between mammalian species, for example between cat and monkey. Here we are interested in examining occurrence of these two response types in the tree shrew, a close relative of primates. Two criteria are commonly used to separate the two cell classes: spatial separation of antagonistic receptive subfields responding to luminance in- and decreases (subfield overlap) and the temporal modulation by a drifting grating stimulus (F1/F0 ratio). Here we quantified both subfield overlap as well as F1/F0 ratio in 120 single neurons recorded from V1 of 16 anesthetized tree shrews. The proportion of “simple” cells found with the two different methods differed dramatically: Using the F1/F0 criterion approximately 42% of neurons were classified as “simple”, whereas using subfield overlap only 7% fell into this category. We argue that this discrepancy is in part explainable by the robust black dominance of a large majority of tree shrew V1 neurons which inflates F1/F0 modulation measures. In a subset of 72 neurons we repeated the measurements after stimulation of the basal forebrain (BF stim), the main source of cholinergic projections to cortex. BF stim led to robust increases in firing rates and significantly reduced F1/F0 ratios ($p<0.01$), such that 19 cells that were classified as “simple” before were now behaving like “complex” cells. Interestingly, the overlap indices were not systematically affected by basal forebrain stimulation ($p>0.1$). Taken together we show that in tree shrew V1, “simple” cells occur rather infrequently and structural receptive field measures like the subfield overlap appear to be more robust to changes in firing rate than the F1/F0 ratio. Our findings suggest that tree shrew V1 does not rely heavily on “simple” cell signals, and these may thus not represent an obligatory step in the transformation of visual information.

Disclosures: J. Veit: None. **A. Bhattacharyya:** None. **R. Kretz:** None. **G. Rainer:** None.

Poster

259. **Striate Cortex: Response Properties I**

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Topic: D.04. Vision

Support: NIH Grant EY02067901

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Title: Spatial tuning for color and luminance in awake area V1

Authors: *M. JANSEN[1], X. LI[1], R. LASHGARI[1], J. KREMKOW[1], Y. BERESHPOLOVA[3], H. SWADLOW[3], Q. ZAIDI[2], J.-M. ALONSO[1,3];
[1]Biol. Sci., [2]Grad. Ctr. for Vision Res., SUNY Col. of Optometry, New York, NY; [3]Psychology, Univ. of Connecticut, Storrs, CT

Abstract: Neurons in primary visual cortex are tuned to different spatial properties of the stimulus such as orientation and spatial frequency. These tuning properties have been extensively studied with achromatic stimuli but systematic comparisons with chromatic stimuli are rare (Johnson et al., 2001, 2008), in part because such comparisons require long recordings from single neurons, which are technically difficult. Here, we took advantage of ultra-thin chronic multielectrode arrays (Swadlow et al., 2005) that allowed us to record single neurons in awake primates for an average time of over 3 hrs. We modulated colors in the cone-based DKL space to generate chromatic red-green (RG) and blue-yellow (BY) gratings. A V1 neuron was classified as 'color neuron' if it responded stronger to chromatic gratings than achromatic gratings with 15% luminance contrast (this contrast value is equal to the maximum cone-contrast in RG gratings and larger than the maximum luminance-contrast artifact caused by the macular pigment in BY gratings). Only 11/42 (26%) were classified as color neurons; the rest were classified as 'luminance neurons'. Compared with luminance neurons, color neurons were less orientation selective (0.22 vs. 0.58, $p<0.05$, t-tests for this and following comparisons) and less sensitive to luminance contrast, both when measured at the contrast that generated the maximum response (90% for color and 61% for luminance, $p<0.05$) and at the half-maximum response (33% for color and 11% for luminance, $p<0.001$). Color and luminance neurons were not different in many other spatial properties including circular variance (0.77 vs. 0.69, $p=0.25$), direction selectivity (0.40 vs. 0.52, $p=0.24$), spatial frequency peak (0.75 vs. 0.82, $p=0.79$ cpd), spatial frequency cut off (0.67 vs. 0.77 cpd, $p=0.57$), low/high spatial frequency ratio (4.00 vs. 2.94, $p=0.63$), grating size that generated maximum response (2.8 vs. 2.3 degrees, $p=0.55$) and half-maximum response (1.3 vs. 1.0 degrees, $p=0.19$), size suppression (0.25 vs. 0.44, $p=0.17$) and phase selectivity (3.68 vs. 2.06, $p=0.17$). Some color neurons responded stronger to intermediate color axes than the sum of the responses to RG and BY axes. Two neurons responded to smaller stimulus sizes for RG than luminance gratings and two other neurons had different spatial frequency tuning for chromatic and achromatic stimuli: one was lowpass for BY and bandpass for luminance; another was lowpass for RG and bandpass for luminance. We conclude that cells representing color and luminance in area V1 have similarly diverse spatial properties but differ in population size, orientation tuning and sensitivity to luminance contrast.

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Poster

259. **Striate Cortex: Response Properties I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 259.13/NN17

Topic: D.04. Vision

Support: Howard Hughes Medical Institute

Title: Convergence of thalamic inputs with multiple receptive fields having different patterns of synchronous spikes optimizes the output reliability and precision of spiny stellate cells in visual cortex

Authors: *C. F. SABOTTKE[1], H.-P. WANG[2], D. J. SPENCER[2], T. J. SEJNOWSKI[2];
[1]Louisiana State Univ., Baton Rouge, LA; [2]Computat. Neurobio. Lab., Salk Inst., La Jolla, CA

Abstract: Synchronous inputs from the lateral geniculate nucleus (LGN) in the thalamus allow visual information from the retina to reliably drive a spiny stellate cell (SSC) in the primary visual cortex (V1) receiving 300-400 thalamic inputs, which is only 5% of all their synaptic inputs. Additionally, LGN and retinal ganglion cells with similar receptive fields produce highly correlated spike trains with millisecond precision, and spike-triggered covariance and related analysis techniques have revealed that V1 neurons have multiple receptive field subunits. We investigated how SSC spike train reliability and precision varied with different patterns of input connections from LGN cells having both similar and different receptive fields. We studied the responses of a multi-compartment model of the SSC, with probabilistic release and short-term synaptic plasticity, to in vivo recordings from adult cat LGN responding to a sinusoidal grating and other visual stimuli. New methods were used to calculate reliability and precision to account for both similar spike patterns and shared periods of silence. We found that there is an optimal range of synchrony that balances high levels of reliability and precision against cellular resource costs. Depending on the connectivity of presynaptic LGN cells to the SSC synapses, different synaptic release patterns emerged that in turn affected the reliability and precision of the SSC spike trains. We found that 40-60 synchronous thalamic synapses maximize spiking reliability and precision, with a broad optimum at around 5-10 synapses per LGN axon, in agreement with anatomical measurements. These findings shed light on how the patterns of connectivity between LGN and V1 affect the reliability and precision of spike trains in the primary visual cortex. We also demonstrate how different combinations of LGN inputs, through synchronous firing, contribute to the receptive field properties of spiny stellate cells.

Disclosures: C.F. Sabottke: None. **H. Wang:** None. **D.J. Spencer:** None. **T.J. Sejnowski:** None.

Poster

259. **Striate Cortex: Response Properties I**

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Topic: D.04. Vision

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National '973' Programs 2009CB941303

Title: Local cues drive population responses to second-order contour stimuli in macaque V1 and V2

Authors: X. AN[1,2], H. GONG[1], J. YIN[1], X. WANG[1], Y. PAN[1], X. ZHANG[1,2], Y. LU[1], Y. YANG[2], Z. TOTH[3], N. MCLOUGHLIN[3], I. SCHIESSL[3], *W. WANG[1];
[1]Vis Percep Mech, Inst. of Neuroscience, State Key Lab. of Neuroscience,CAS, Shanghai, China; [2]Key Lab. of Brain Function and Diseases, Sch. of Life Sciences, Univ. of Sci. and Technol. of China, Hefei, China; [3]Fac. of Life Science, Univ. of Manchester, Manchester, United Kingdom

Abstract: In primate V1 and V2 most neurons with small spatio-temporal receptive fields respond selectively to oriented luminance contours, while only a subgroup of neurons signals non-luminance defined contours (second-order). These properties raise the question whether population responses of V1 and V2 mainly reflect the global representation of second-order contours or the processing of the local physical inducers that define second-order contours? Here we compared the population responses of macaque V1 and V2 to three types of second-order contour stimuli generated either by motion (kinetic contours), modulation of contrast, or phase reversal. We found that, both with intrinsic optical imaging and spatio-temporal energy model simulation, the local visual cues within these second-order contour stimuli drove the population responses within the orientation columns of both macaque V1 and V2. These results suggest that the primate early visual system initially makes use of local luminance and motion cues for processing the orientation of second-order contours, prior to form-cue invariant shape processing in higher-tier visual cortices. Our results of population responses to second-order contour stimuli defined by first-order inducers also suggest that the orientation maps within primate V1 and V2 can be described as a spatial-temporal energy map.

Disclosures: X. An: None. **H. Gong:** None. **J. Yin:** None. **X. Wang:** None. **Y. Pan:** None. **X. Zhang:** None. **Y. Lu:** None. **Y. Yang:** None. **Z. Toth:** None. **N. McLoughlin:** None. **I. Schiessl:** None. **W. Wang:** None.

Poster

259. Striate Cortex: Response Properties I

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 259.15/OO1

Topic: D.04. Vision

Support: Max-Planck Society

Bernstein Center for Computational Neuroscience, Tuebingen (BMBF; FKZ:01GQ1002)

Title: Stimulus-dependent gamma-frequency shifting in the macaque v1

Authors: *K. LAKSHMINARASIMHAN[1,2], N. K. LOGOTHETIS[2,3], G. A. KELIRIS[2,4]; [1]Dept. of Neurosci., Baylor Col. of Med., Houston, TX; [2]Max-Planck Inst. for Biol. Cybernetics, Tuebingen, Germany; [3]Imaging Sci. and Biomed. Engin., Univ. of Manchester, Manchester, United Kingdom; [4]Bernstein Ctr. for Computat. Neurosci., Tuebingen, Germany

Abstract: The phase of spikes in the gamma cycle has previously been shown to be modulated by stimulus orientation in macaque V1 (Vinck et al. 2010, Womelsdorf et al. 2012). These studies suggested that such stimulus-dependent phase shifts selectively facilitate the impact of neurons that fire earlier in the gamma cycle, on their targets. However, such phase coding schemes implicitly depend on the generation of a consistent gamma oscillation frequency across stimulus conditions. To test this, we examined whether the stimulus orientation and the eye of presentation affected the peak gamma frequency in V1. Two macaque monkeys were trained to passively fixate on a central spot, while one of two orthogonally oriented gratings was presented monocularly through a mirror stereoscope for a period of one second. In different trials either the eye of presentation or the orientation, were changed. Local field potential (LFP) signals recorded from 168 sites across multiple sessions were found to exhibit significant coherence with concurrently recorded single-unit spikes in the gamma frequency range. The power spectral density of each of those LFPs was fit as the sum of a power function and a gaussian function, and the center of the gaussian was taken as the peak gamma frequency. We found that, across sites the peak frequency varied between 30 Hz and 45 Hz in both monkeys. Moreover, within each site there was a significant shift in the peak frequency both with orientation (median shift ~1.99Hz) as well as the eye of presentation (median shift ~0.89Hz). There was no systematic relationship between the direction of shift and stimulus preference. Given that the orientation-dependent phase shifts reported earlier were of a very small magnitude (only a few degrees), it follows that such frequency changes could be detrimental for phase-shift coding schemes that rely on entrainment of multiple cortical areas to a single ‘clock-like’ signal. Alternatively, neural

mechanisms implementing phase computations could be relatively invariant to frequency changes by using more intricate signal properties like instantaneous phase.

Disclosures: K. Lakshminarasimhan: None. **N.K. Logothetis:** None. **G.A. Keliris:** None.

Poster

259. **Striate Cortex: Response Properties I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 259.16/OO2

Topic: D.04. Vision

Support: FACETS-ITN Marie-Curie grant

Title: Motion integration along a trajectory by neuronal population in alert monkey V1

Authors: *G. BENVENUTI, G. S. MASSON, F. CHAVANE;
INT, CNRS, Marseille, France

Abstract: We have recently shown that a bar approaching the RF of a V1 neuron along a trajectory can generate an “anticipatory” response that builds up gradually with time before the bar actually enters into the classical RF of the cell and modulates the response within the RF (Benvenuti et al. 2011, SfN). This study was conducted in alert monkeys over a population of 83 V1 neurons (SUA), recorded in different sessions, in response to a bar moving along straight trajectories of different length before entering the RF center. We proposed that this "anticipation" results from the integration of convergent propagations of activity dynamically recruited along the neuronal population that is getting gradually activated by the retinal input.
Using a similar experimental paradigm, we further studied this trajectory-dependent response at the level of V1 population of alert fixating monkey chronically implanted with multi-unit UTAH array (96 electrodes on a squared matrix of 4mm). It gave us the opportunity to record simultaneously single-unit, multi-unit and LFP from a population of neurons with partially superimposed RFs and different tunings properties over multiple recording sessions. The spatial profile and tuning properties of the recorded neurons’ RFs was first carefully characterized using sparse noise stimuli and local gratings. We then presented a bar moving towards and across the RFs with different trajectory lengths (1.5 ,3 ,6°). Over many sessions, we have investigated the dependence of the trajectory-dependent response to several stimulus parameters such as: bar directions (0, 90 ,135 ,180 ,225 ,270°), bar orientations (30, 60, 90, 120 and 150° relative to motion direction), speeds (2 ,6.6 ,12 ,16 ,19°/s) and bar lengths (1, 2, 4 , 8°). Depending on the stimulation configuration, RFs could be crossed one after the other by the bar, or at the same time, or none when we used a local mask over the population RF. Our results suggest that this

trajectory-dependent response is a contextual priming signal that influences the detection and the identification of various properties of the moving stimulus.

Disclosures: G. Benvenuti: None. **G.S. Masson:** None. **F. Chavane:** None.

Poster

259. **Striate Cortex: Response Properties I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 259.17/OO3

Topic: D.04. Vision

Support: CIHR Grant MA-9685

CIHR Grant MOP-119498

Title: Three distinct types of simple cells in visual cortex: Spatiotemporal receptive field properties derived from natural images

Authors: *V. TALEBI, C. L. BAKER;
McGill Vision Res. Unit, McGill Univ., Montreal, QC, Canada

Abstract: The retina and lateral geniculate nucleus (LGN) have many categorically distinct types of neurons, but in the visual cortex, simple and complex cells are the only well-defined classes. However our recent work using natural image stimuli to estimate a linear-nonlinear (LN) model has revealed three distinct classes of early cortical simple cells, based on their spatial receptive field (RF) structure and power law output nonlinearity. Here we quantitatively examine the spatiotemporal response properties of these three categories, to gain insight into their functional roles. Extracellular recordings were made from simple-type cells in area 18 (A18) of anesthetized, paralyzed cats in response to natural images. We utilized a primary dataset to estimate the 3D spatiotemporal RF, and independent hold-back datasets for regularization and predictive evaluation. A gradient descent, iterative regression algorithm with regularization was employed to estimate a linear model of the RF, and a subsequent zero-memory nonlinearity (half-wave rectifier and power law) was fit to the data. Estimated RF models were analyzed to characterize their spatial tuning, temporal dynamics, spatiotemporal behaviour, and spiking properties. The "non-oriented" RFs lacked orientation and direction selectivity, and had higher spatial frequencies and shorter temporal durations, suggesting that they might be more directly driven by LGN afferents. The other two categories of RFs were selective for orientation and often, direction of motion. The "expansive-oriented" cells differed from the "compressive-oriented" cells not only in shape of the output nonlinearity, but also in their responsivity and

degree of correspondence to an LN model, suggesting that they differed fundamentally in the degree to which a nonlinear gain control mechanism was engaged. The three classes also differed in the extent to which they spanned complementary ranges of temporal latencies and durations. Results so far suggest that each of the classes might be further subdivided on the basis of optimal spatial frequency. Other RF properties such as spatial frequency bandwidth, optimal orientation, aspect ratio, and RF ON/OFF dominance did not bear any relationship to the three categories. This work challenges the prevailing idea that due to the rich connectivity within cortical circuitry, each neuron will show some physiological attributes of many others, thus blending them together into a multi-dimensional continuum of RF properties. Rather, response properties in cortex are categorically distinct, implying that cortical neurons may have specialized functional roles.

Disclosures: V. Talebi: None. **C.L. Baker:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.01/OO4

Topic: D.04. Vision

Support: NIH R21EY018925

NIH R01EY015980

NIH R01EY019466

NIH R01AG031941

NIH R01MH091801

NSF BCS-0964776

NCRR P41RR14075, S10RR021110

Title: Role of sleep spindle in early visual areas and perceptual learning

Authors: *J. BANG, T. WATANABE, Y. SASAKI;
Brown Univ., Providence, RI

Abstract: Sleep is known to consolidate memory. However, the underlying neural mechanism has yet to be completely understood. Here, to better understand the mechanism for the

facilitatory effect of sleep on visual perceptual learning, we measured fine-scaled spatio-temporal neural activity during sleep after training of texture discrimination task (TDT) using a multimodal neuroimaging technique that combines MEG and MRI. A leading hypothesis suggests that the sleep spindle activity during the non-rapid eye movement sleep (NREM) is involved in the facilitatory effect. Since the TDT is associated with changes in the region of the early visual areas that retinotopically corresponds to the trained visual field quadrant, we tested whether the strength of sleep spindles in early visual areas is correlated with the facilitatory effect during sleep on TDT.
Young and healthy participants underwent an MRI session after 3 nightly MEG sessions including 1 adaptation, pre-, and post-training sleep. Before the post-training sleep, TDT was conducted twice; the initial test and intensive training in one visual field quadrant. After the post-training sleep, we conducted a re-test of TDT. Wavelet-transformed MEG during sleep was combined with high-resolution MRI to constrain the current locations to the cortical mantle individually. We measured the strength of the sigma activity that represents sleep spindles in the 2 cortical parts in early visual areas which retinotopically corresponds to the trained and untrained visual field quadrants, based on the retinotopic mapping. The results showed that sigma activity yielded greater power increase in the trained region of early visual areas than in the untrained region of early visual areas after training. In addition, the power change of sigma activity was significantly correlated with the learning amount in TDT. This suggests the feasibility of the hypothesis that the sleep spindle activity is involved in the consolidation of TDT during sleep.

Disclosures: J. Bang: None. **T. Watanabe:** None. **Y. Sasaki:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.02/OO5

Topic: D.04. Vision

Support: Commonwealth of Pennsylvania tobacco funds

Title: Development of GABAergic neuron response properties in visual cortex in-vivo

Authors: *S. J. KUHLMAN, R. ZHANG;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Cortical GABAergic inhibition is a key mediator of visual development, and accumulating evidence identifies aberrant GABAergic function in neurodevelopmental disease.

However, the mechanisms by which inhibition regulates experience-dependent development of vision are not well-defined, nor is it understood how disturbance of cellular signaling pathways within inhibitory interneurons impacts GABAergic circuit assembly in terms of recruitment of inhibition in-vivo. Using in-vivo targeted electrophysiological recording of an identified inhibitory interneuron cell type, the parvalbumin (PV+) fast-spiking GABAergic interneuron, we demonstrated that visual experience uniquely broadens orientation tuning of layer 2/3 PV+ interneurons (Kuhlman et al., Nat Neurosci 2011). Our results support a model in which PV+ broadening is mediated by the development of excitatory synapses onto PV+ interneurons.
We are currently testing the hypothesis that increased local recurrent feedback from excitatory neurons within layer2/3 is a critical step in the maturation of PV+ response properties, and are defining the role of tyrosine kinase signaling and AMPA receptor trafficking in this process. Response properties to visual stimulation are assayed using patch-clamp electrophysiology and gCamp6 calcium imaging in vivo.
Identification of the molecular pathways that mediate PV+ maturation and integration into the circuit will facilitate the generation mouse models in which maturation of PV+ neurons is either accelerated or stunted; the impact of aberrant recruitment of inhibition on sensory learning and new skill acquisition will be studied in go/no-go discrimination tasks.

Disclosures: S.J. Kuhlman: None. **R. Zhang:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.03/OO6

Topic: D.04. Vision

Support: CIHR, Elvire Vaucher, MOP-111003

Title: Combined repetitive Donepezil administration and visual exposure increases visual acuity in rats

Authors: *M. CHAMOUN, J. IL KANG, F. HUPPÉ-GOURGUES, E. VAUCHER;
Optometrie, Univ. De Montreal, Montreal, QC, Canada

Abstract: Memory and learning are associated with cortical release of acetylcholine(ACh). Rat's visual acuity and cortical activity for a specific pattern can be improved when repeated visual exposure of a specific stimulus is paired with electrical stimulation of basal forebrain cholinergic neurons. The clinical approach to stimulate the cholinergic transmission in human consists in increasing extracellular ACh level by acetylcholinesterase inhibitor (AChEI), the degradation

enzyme for ACh. Donepezil is a non-competitive and reversible AChEI which has been shown to cause significant increase of ACh concentration in humans as well as in rats. In addition, Donepezil induces beneficial effects on learning performance in rats.
In the present study we evaluated whether repeated visual exposure of a specific stimulus would also change the visual acuity of the rats for the trained stimulus when the exposure is paired with Donepezil administration. Visual evoked potentials (VEPs) to a sinusoidal phase converting grating pattern, shown in a pseudo-random manner for 30° and 120° orientations and different spatial frequencies (0.08 to 1 CPD), were recorded in rat's primary visual cortex (V1) before and after the two weeks visual exposure. The two weeks visual exposure consisted in 10 min daily exposure to a 0.12 cycle per degree (CPD) 30° sinusoidal pattern, in rats injected with Donepezil or saline 20 min prior to visual exposure. An index of variation (Ivep = post-exposure VEP amplitude - pre-exposure VEP amplitude) was then calculated to determine the variation of the cortical response.
Our results show that VEPs index of variation for the 30° orientation was significantly increased in Donepezil injected rats at high spatial frequencies (0.9 CPD: 0.5±0.18, Mann-Whitney p=0.027 and 1.0 CPD: 0.45± 0.2 Mann-Whitney p=0.050) but not in control animals (0.9 CPD: -0.15±0.14, 1.0 CPD: -0.20±0.13). The VEP amplitude for the 30° orientation showed an increasing tendency at the training frequency 0.12 CPD, (0.64 ± 0.39, Mann-Whitney p=0.64) but not in the control group (-0.04 ± 1.3). No significant difference was observed in the post- vs pre- VEPs amplitude to any spatial frequency for the 120° orientation.
Our results showed increased visual acuity in rats trained with Donepezil. This agrees with the previous study in which the cholinergic system stimulation was provided by electrical stimulation of the basal forebrain. Overall, we demonstrated the possibility to increase visual performance and cortical response by combination of cholinergic stimulation and visual exposure in rats.

Disclosures: M. Chamoun: None. **J. Il Kang:** None. **F. Huppé-Gourgues:** None. **E. Vaucher:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.04/OO7

Topic: D.04. Vision

Title: Environmental experience and visual system development and plasticity: Epigenetic effects

Authors: *L. BARONCELLI[1], I. MANNO[2], M. SCALI[1], G. SANSEVERO[1], L. MAFFEI[1,2], A. SALE[1];
[1]Neurosci. Institute, CNR, Pisa, Italy; [2]Scuola Normale Superiore, Pisa, Italy

Abstract: Despite more than 50 years of research on the effects of environmental enrichment (EE) on the brain, very few studies have investigated the influence of enhanced sensory-motor experience on the development and plasticity of sensory cortices. Recently, a new approach in which the traditionally separated fields of EE and manipulation of visual experience have been combined together has shown that early EE robustly accelerates the development of the visual system, and that exposure to EE in adulthood enhances visual cortex plasticity well after the end of the critical period. While some critical factors underlying these effects have already emerged, it remains unknown whether changes at the level of chromatin structure and function are also involved as possible mechanisms underlying the effects of EE. Here, we report that histone acetylation is enhanced both in the visual cortex of preweaning pups raised in enriched conditions, in parallel with an accelerated time course of the critical period for ocular dominance plasticity, and in the visual cortex of adult rats in which ocular dominance plasticity is reinstated by exposure to EE. These findings suggest an involvement of the epigenetic machinery as a possible mediator of the impact of EE on visual system development and plasticity.

Disclosures: L. Baroncelli: None. **I. Manno:** None. **M. Scali:** None. **G. Sansevero:** None. **L. Maffei:** None. **A. Sale:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.05/OO8

Topic: D.04. Vision

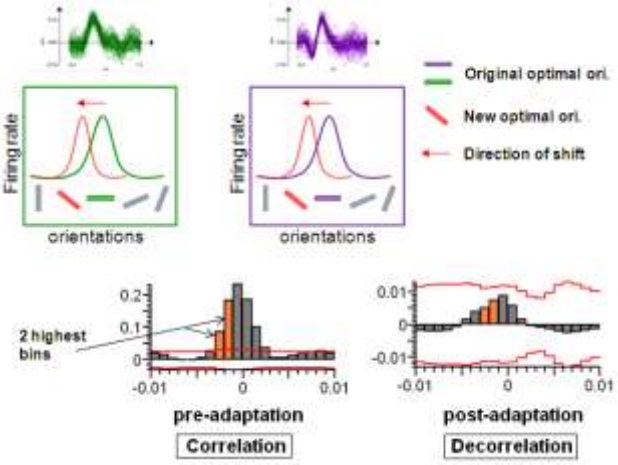
Support: CRSNG

FQRNT

Title: Correlating neuronal activity after adaptation-induced-plasticity in V1

Authors: *L. BACHATENE, V. BHARMAURIA, S. CATTAN, J. JEYABALARATNAM, S. MOLOTCHNIKOFF;
Dept. of Biol. Sci., Univ. De Montréal, Montréal, QC, Canada

Abstract: The primary visual cortex has the ability to adapt to modified environment features in order to acquire an unfamiliar property of the visual scene. In adult mammals, experience dependant plasticity within the orientation columns of primary visual cortex shifts the tuning curves of neurons which allows the adjustment of their selectivity. Perceptual adaptation to a non preferred oriented stimulus leads to such shifts. These plastic mechanisms may involve alterations in the synaptic weights between cells for the presented oriented stimuli potentiating change in orientation preference at the neuronal level. Thus the question arises: how these connectivity weights are modulated between pairs of neurons which share the same preference properties pre and post visual adaptation, i.e. how the connection probabilities vary after orientation-induced-plasticity?
In the present experiments, we used cross correlation between the trains of action potential of neuronal pairs in order to disclose the modifications of the probabilities of connections between cells before and after visual adaptation. We used electrophysiological recordings in primary visual cortex of anaesthetized adult cats that were visually stimulated by presenting sine-wave drifting oriented gratings. A non preferred orientation was presented for a prolonged duration (12 min) to promote visual adaptation. Cell pairs were selected based on the similarity of their original optimal orientation (±22 deg.) and the shift post adaptation. Shifted and subtracted crosscorrelograms were constructed between the trains of spikes of the cell pairs, and the two highest adjacent bins within ±5ms time window of the crosscorrelogram were compared pre and post adaptation to compare the probability of connection of neurons and its modulation. Results indicate a decorrelation of connection probabilities for the original optimal orientation as well as for the new acquired preferred orientation after adaptation which suggests a recalibration of connectivity strength when the modifications of orientation selectivity are occurring.



Disclosures: L. Bachatene: None. **V. Bharmauria:** None. **S. Cattan:** None. **S. Molotchnikoff:** None. **J. Jeyabalaratnam:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.06/OO9

Topic: D.04. Vision

Support: CRSNG

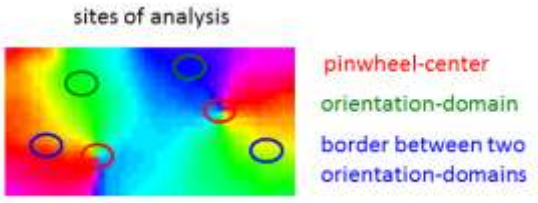
FQRNT

CERNEC

Title: Orientation-domains exhibit more plasticity than pinwheel-centers in V1

Authors: *S. CATTAN[1], V. BHARMAURIA[1], L. BACHATENE[1], J. JEYABALARATNAM[1], J. RIBOT[2], C. MILLERET[2], S. MOLOTCHNIKOFF[1];
[1]Sci. Biologiques, Univ. De Montréal, Montréal, QC, Canada; [2]Lab. de Physiologie de la Perception et de l'Action, Collège de France, Paris, France

Abstract: Object-orientations in the visual field are columned into specific orientation-domains in the primary visual cortex (V1), where neurons in one cortical column respond preferentially to a determined orientation. It has previously been shown that adapting V1 neurons for variable durations of time (Ghisovan et al., PLoS ONE 2008; Bao and Engel, Proc Natl Acad Sci U S A 2012; Bachatene et al., Eur J Neurosci 20013; Jeyabalaratnam et al., PLos ONE in press) induces shifts in neurons' original preferred orientation. Previous studies (Dragoi et al., Neuron 2001; Schummers et al., Prog Brain Res 2005) reported that neurons located at pinwheel-centers are more susceptible to adaptation, and display larger shifts than cells located within orientation-domains. We re-examined these results by comparing neuronal activity on optical-imaging-maps (i.e. mean activity of 0.38 mm² cortical areas corresponding to one pixel) before and after adaptation in anesthetized cats at three locations: in pinwheel-centers, at the junction of two orientation-domains and inside orientation-domains (see figure). Results indicate that shifts in pinwheel-centers are smaller than those in borders between two orientation-domains. In orientation-domains the shifts are largest.
We hypothesize that since neurons in pinwheel-centers have direct relationships with neurons tuned to different orientations, their orientation-selectivity post-adaptation can be attributed to either: 1). neuronal orientation-selectivity in pinwheels is less likely to be changed by neighboring neurons as the synaptic-links between them are weaker, consequently, the amplitude of shifts is smaller, or 2). since many orientations are interspersed in pinwheel, they are likely to get input from many orientations, thus resulting in higher variance of shift-amplitudes. Since our results show smaller shifts in pinwheel-centers, we conclude that our first hypothesis is more likely suggestible for conservation of orientation-maps at these areas of singularity.



Disclosures: S. Cattan: None. **V. Bharmauria:** None. **L. Bachatene:** None. **J. Jeyabalaratnam:** None. **S. Molotchnikoff:** None. **J. Ribot:** None. **C. Milleret:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.07/OO10

Topic: D.04. Vision

Support: RO1-EY017656-05

Title: *In vivo* imaging of coordinated excitatory and inhibitory synaptic dynamics on pyramidal cell dendrites

Authors: *K. L. VILLA, K. P. BERRY, J. SUBRAMANIAN, J. CHA, P. T. C. SO, E. NEDIVI;
MIT, Cambridge, MA

Abstract: Inhibitory circuit development is important for defining critical period windows during development, and inhibitory cells continue to exert effects on experience-dependent cortical plasticity into adulthood. A common theme of neuronal plasticity is the capacity for dynamic adaptations to ever changing environments. One way this can be accomplished is through the structural remodelling of synapses and dendrites. Recent in vivo imaging studies demonstrate that long-term plasticity of inhibitory circuits can be manifested through structural rearrangements. Alterations in sensory experience drive structural remodelling of inhibitory interneurons in a cell type and circuit specific manner. Recently, we found that inhibitory synapse formation and elimination occurs with a great deal of spatial and temporal precision and that these events are often paired with excitatory synapse alterations within 10um on the same dendrite. To probe the nature of this ‘coordination’ between inhibitory and excitatory synapse dynamics, we triple-labelled L2/3 pyramidal neurons in the mouse visual cortex using YFP as a cell fill, PSD95-mcherry as a post synaptic excitatory synapse marker, and Teal-Gephyrin as a

post synaptic inhibitory synapse marker via in utero electroporation. These mice were implanted with cranial windows as adults and labelled neurons in the binocular visual cortex were imaged in vivo at short intervals using spectrally resolved two-photon microscopy. Surprisingly, we observed increased dynamics when imaging at these shorter intervals as compared to previous 4 day imaging intervals. Here we explore the kinetics, distribution, and probabilities of these dynamic events.

Disclosures: K.L. Villa: None. **K.P. Berry:** None. **J. Subramanian:** None. **J. Cha:** None. **P.T.C. So:** None. **E. Nedivi:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.08/OO11

Topic: D.04. Vision

Support: CIHR, EV, MOP-111003

Title: Cortical enhancement induced by pairing cholinergic system and repetitive exposure to visual stimulus is mediated by GABAergic modulation

Authors: *J. KANG, F. HUPPE-GOURGUES, E. VAUCHER;
Sch. Optometry, Montreal Univ., Montreal, QC, Canada

Abstract: Daily pairing cholinergic system activation and visual stimulation with a specific pattern during 2 weeks induced an improvement of the visual acuity for this specific pattern as well as an increase of the cortical response of the primary visual cortex (V1). This process involves both glutamatergic and GABAergic local V1 neurons. In the present study, we investigated the involvement of the GABAergic system in these mechanisms that mediate the visual enhancement. The visual acuity and cortical response were measured by recording the visual evoked potentials (VEP) in V1 before and after the visual training combined with cholinergic system stimulation and local administration of GABAA receptors agonists or antagonists.
We recorded 40 field potentials on an isoflurane anesthetized rat. Visual stimulation consisted of sinusoidal grating pattern phase converting and shown in a pseudo-random manner for each orientation (30° and 120°) and spatial frequency (0.08 to 1 CPD). Pre- and post-training cortical responses (signal/baseline) were measured and compared in the V1. An index of variation (IVEP = post-exposure VEP amplitude - pre-exposure VEP amplitude) was then calculated to determine the variation of the cortical response. The visual training was performed on restrained awaken

rats during 10min/day for 7 days. Visual system activation (phase converting sinusoidal gratings at 0.12 CPD; 30°orientation) was displayed on 3 computer monitors. During visual stimulation, cholinergic system was electrically stimulated through an electrode previously implanted in the basal forebrain (BF). GABAergic agonist, muscimol (200µM) or GABAergic antagonist picrotoxin (100µM) was also infused during visual training through a pre-implanted push-pull cannula (i.c.) in the V1.
VS/BF stimulated group showed a specific increase of cortical response for the trained stimulus (1.81 ± 0.57, one-way ANOVA, post-hoc LSD, p=0.023) compared to control group (0.11 ± 0.44). No other spatial frequency than 0.12 CPD were significantly changed during 1 week of visual training. Muscimol injected group also showed no significant increase of VEP for 0.12CPD (0.15 ± 0.45) after training but picrotoxin injection during exposure induced an amplification of VEP increase (2.8 ± 1.1, p=0.003).
Our study demonstrates that the repeated visual exposure paired with cholinergic stimulation induces increase of cortical response. Also this increase can be abolished by GABA activation but GABAA receptor inhibition can enhance the cholinergic stimulation effect. Overall, these results indicate that the GABAergic system contributes to cholinergic-dependent plasticity.

Disclosures: J. Kang: None. **F. Huppe-Gourgues:** None. **E. Vaucher:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.09/OO12

Topic: D.04. Vision

Support: NIH Grant R01EY011894-15

Title: Molecular specificity for synapse and spine stabilization in layer II/III pyramidal neurons in visual cortex

Authors: *J. SUBRAMANIAN[1], J. CHA[2], P. SO[3], E. NEDIVI[4];
[2]Dept. of Mechanical Engin., [3]Dept. of Mechanical Engineering. Dept. of Biol. Engin., [4]Picower Inst. for Learning and Memory, Dept. of Biology, Deparment of Brain and Cognitive S, [1]MIT, Cambridge, MA

Abstract: The temporal sequence and mechanisms underlying formation and stability of dendritic spines in relation to their synaptic inputs are poorly understood. To simultaneously track formation and elimination of dendritic spines as well as excitatory and inhibitory inputs onto these spines, we developed a multi-color, two photon microscopy system for in vivo

imaging of entire layer 2/3 pyramidal neuron arbor in adult mouse visual cortex. We find that synapse formation and elimination are protracted processes that occur over a period of days. PSD95, a post-synaptic density protein is recruited to pre-existing dendritic spines and that the presence of PSD95 correlates with spine stability. We further show that CPG15, a molecule known to promote synapse maturation and stabilization mediates PSD95 recruitment to dendritic spine excitatory synapses, but is not required for inhibitory synapse formation. In the absence of CPG15, the density of mature dendritic spines was significantly lower. Fewer new spines were able to recruit PSD95, and the loss of spines lacking PSD95 was markedly increased. In contrast, the stability and the density of inhibitory synapses or dendritic spines innervated by them remained unaltered. Thus, dually (excitatory + inhibitory) innervated spines were comparable in their dynamics between WT and CPG15 knockout animals. Our data demonstrates distinct requirements for stabilization of excitatory and inhibitory synapses in vivo. Our data further supports a role for CPG15 in synapse maturation through stabilization of nascent spines and or synapses, and shows that synapse formation is a driving force in dendritic spine stabilization, irrespective of the synapse type.

Disclosures: J. Subramanian: None. **J. Cha:** None. **P. So:** None. **E. Nedivi:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.10/OO13

Topic: D.04. Vision

Support: NIH EY-014645

Title: Population receptive field analysis of auditory frequency tuning in early blind individuals

Authors: *E. HUBER, J. M. THOMAS, I. FINE;
Univ. of Washington, Seattle, WA

Abstract: Introduction: Early onset blindness has been linked to enhanced auditory abilities and altered BOLD responses in both auditory and occipital cortex. In auditory cortex, changes in both the amplitude and extent of responses have been noted (Elbert et al., 2002; Stevens and Weaver, 2009). In occipital cortex, early blind subjects show cross-modal responses to a wide range of auditory stimuli across multiple visual areas (e.g., Poirier et al., 2006; Roder et al., 2002; Voss et al., 2008). Here, we measured tonotopic organization in early blind and sighted subjects to assess group differences in tonotopy and characterize the tuning properties of cross-modal responses to pure tones in early blind subjects. Methods: We carried out tonotopic

mapping in auditory and occipital cortex in early blind and age-matched sighted controls using functional magnetic resonance imaging. Our stimuli were pure tones ranging from 88 to 8000 Hz, presented in randomized sequences during six separate runs in a single scanning session. Data were analyzed using an adaptation of the population receptive field technique developed by Dumoulin and Wandell (2008). Our model treats the aggregate receptive field underlying each voxel's response as a one-dimensional Gaussian function of frequency. This technique provides an estimated sensitivity function for each voxel with a given center, or preferred frequency, and standard deviation which describes the population tuning bandwidth. Results: We obtained consistent and reliable tonotopic maps within auditory cortex for both subject groups. It remains to be determined with additional subjects whether tuning bandwidth and/or the size of PAC might differ across blind and sighted subjects. We also observed cross-modal responses to pure tones in early blind, but not sighted, subjects. Frequency selective population receptive field estimates in occipital cortex of blind subjects were as robust as estimates in auditory cortex. The majority of these occipital voxels were narrowly tuned to frequencies within a 1000-1500 Hz range. Conclusions: Population receptive field estimates suggest that the cortical mapping of frequencies in auditory cortex follows a similar organization in sighted and blind individuals. In addition, the distribution of preferred frequencies in occipital cortex of blind subjects suggests a greater representation in the range of 1000-1500 Hz, which may provide a basis for superior performance of various auditory tasks.

Disclosures: E. Huber: None. **J.M. Thomas:** None. **I. Fine:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.11/OO14-DP8

Topic: D.04. Vision

Support: The Wellcome Trust C2D2 Award, University of York

Title: The involvement of early visual cortex in tactile discrimination of Braille and visual discriminations of letters in a patient with low vision

Authors: *A. D. GOUWS[1], E. H. SILSON[1], K. L. HAAK[1], J. RODGERS[1], M. CROUCHER[1], M. HYMERS[1], H. A. BASELER[1,2], G. E. LEGGE[3], A. B. MORLAND[1,2];
[1]YNiC, Univ. of York, York, United Kingdom; [2]Ctr. for Neurosci., Hull-York Med. Sch., York, United Kingdom; [3]Psychology, Univ. of Minnesota, Minneapolis, MN

Abstract: Patient S has low vision and can read Braille. Previous research on S has shown greater activity to Braille than visual stimuli at the occipital pole and lateral occipital areas, a pattern not found in normally sighted individuals (Cheung et al, Current Biology, 2009, 19, 596-601). The occipital pole normally forms part of early visual areas including V1. This study aimed to examine the causal role played by the occipital pole in S when discriminating letters, in both the tactile (Braille) and visual domains. We used fMRI to identify regions of the brain that had greater responses to Braille than to visual stimuli and vice versa. As previously, the results highlighted Braille selective responses at the occipital pole. As expected, controls only exhibited visually selective responses at the occipital pole. Both controls and S exhibited Braille selective responses in somatosensory cortex (S1). We used the outcome of the fMRI experiment to generate targets for TMS. Specifically, we defined (1) an occipital pole target (OP) as the most medial aspect of Braille selective cortex, (2) a target located in Braille selective cortex of S1 (S1). Targets were selected in the hemisphere contralateral to the Braille reading finger. In psychophysical experiments we then presented four Braille letters (A, L, Q, X) on a computer controlled Braille character array. All pins in the array were displaced for 200ms and the letter was defined by additional pin displacements. We established pin displacement thresholds for Braille letter discrimination (60 % correct) in a four alternative forced choice task. In similar forced choice experiments we established the size and contrast of the same letters presented visually. TMS pulses were delivered during stimulus presentation to OP and S1 while S performed tactile and visual letter discriminations of stimuli presented at his thresholds. In S, visual discrimination was strongly affected following stimulation of OP, but not S1. In contrast, stimulation of S1, but not OP, resulted in poor Braille discrimination. This double dissociation was entirely consistent with control data. It appears therefore that while fMRI shows that OP is Braille selective, it does not appear to play a causal role in S's ability to perform tactile tasks. It is possible that foveal representations in early visual areas at the occipital pole OP receive top-down input from extrastriate visual areas that are causally involved in Braille tasks. Further TMS experiments that probe the causal role of more lateral, extrastriate regions of Braille selective cortex in S are therefore warranted.

Disclosures: A.D. Gouws: None. **E.H. Silson:** None. **K.L. Haak:** None. **J. Rodgers:** None. **M. Croucher:** None. **M. Hymers:** None. **H.A. Baseler:** None. **G.E. Legge:** None. **A.B. Morland:** None.

Poster

260. **Striate Cortex: Plasticity**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 260.12/OO15

Topic: D.04. Vision

Title: The effects of retinal degeneration on crossmodal sensory takeover of the visual cortex

Authors: *M. K. JARVINEN, R. MORROW, B. SILVEIRA, J. MCEWEN;
Emmanuel Col., Boston, MA

Abstract: One fundamental principle of neocortex organization is the localization of functions to specific neural structures. The visual cortex is an excellent example of this principle. In rodents, the onset of the critical period that leads to mature visual function is well underway by postnatal day (PND) 28 and declines after PND 35. However, much less is known about the timeline and mechanisms involved in the structural reorganization of the visual cortex, particularly after naturally-occurring visual deafferentation. We previously identified when vision loss occurred in a mouse model of retinal degeneration (rd). By PND 49, the loss of vision corresponded with altered gene expression and cell population densities in the visual cortex. The main objective of this current study was to evaluate whether structural reorganization was evident in the visual cortex of rd mice and, if so, to identify the timeline and extent by which it occurred. For our pilot study, we sampled rd mice at PND 49 and randomly assigned them to two different groups. Group 1 (N=6) received auditory/tactile (80dB white noise + focused air flow from small fan) and Group 2 (N=6) received no crossmodal stimulation. After 90 minutes of sensory exposure, all animals were sacrificed, the visual cortices were harvested, and brain sections were processed using c-fos immunohistochemistry. Neurons that were above background threshold were quantified using image analysis software. We found a 15% increase in the number of c-fos labeled neurons in the visual cortex in Group 1 rd mice compared to Group 2 controls. We are currently validating these findings by using sensory stimulation kits from SR-Lab behavioral testing apparati to provide focal and systematic stimulation of the various sensory pathways. Our current data suggest that retinal degeneration modifies sensory wiring in the visual system at a time during development that may result in significant crossmodal sensory takeover. These active neurons may be functionally relevant and provide a mechanism for enhanced sensory detection.

Disclosures: M.K. Jarvinen: None. **R. Morrow:** None. **B. Silveira:** None. **J. McEwen:** None.

Poster

261. **Spatial Attention**

Location: Halls B-H

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Program#/Poster#: 261.01/OO16

Topic: D.04. Vision

Support: NIH Grant R21 MH094938

Title: Neural activity in a midbrain cholinergic nucleus, the Ipc, during attention-demanding tasks in chickens

Authors: *J. SCHWARZ, D. SRIDHARAN, E. I. KNUDSEN;
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Abstract: The rapid and reliable selection of relevant stimuli (targets) for gaze and attention is essential for survival in complex environments. This process is governed by interactions within and between networks in the forebrain and the midbrain. Among vertebrates, the midbrain network is most differentiated in birds: the optic tectum is functionally segregated into 15 distinct layers, and the interconnected isthmic nuclei in the tegmentum are clustered into distinct nuclei. This midbrain network is thought to be involved in selecting stimuli for attention as well as targets for gaze shift. The *isthmi pars parvocellularis* (Ipc, homologous to the mammalian parabigeminal nuclues) is a cholinergic tegmental nucleus that connects reciprocally and homotopically with the optic tectum. The precise role of the Ipc in the process of selection remains unknown. We have developed a system for recording neural activity in the Ipc of domestic chickens (*Gallus gallus domesticus*) performing tasks of spatially cued localization and orientation discrimination. We observed that the Ipc stimulus-driven activity was modulated powerfully by a spatial top-down cue. In addition, it conveys gamma-modulated activity that reliably predicts the target of impending shifts of gaze. These results suggest that the Ipc participates in the selection of targets for gaze and attention, and support the hypothesis that this cholinergic nucleus is a critical node in controlling the locus of spatial attention.

Disclosures: J. Schwarz: None. **D. Sridharan:** None. **E.I. Knudsen:** None.

Poster

261. **Spatial Attention**

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Program#/Poster#: 261.02/OO17

Topic: D.04. Vision

Title: Beta/gamma cross-frequency interaction as a mechanism of top-down attentional bias

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Main, Germany; [3]Karolinska Inst., Stockholm, Sweden; [4]Univ. of Amsterdam, Amsterdam, Netherlands; [5]Donders Inst. for Brain, Cognition and Behaviour, Nijmegen, Netherlands

Abstract: The neural mechanisms of top-down control, which are required for the implementation of processes such as selective attention, have remained poorly understood within the neurosciences for decades. Though fronto-parietal structures are believed to govern the control of spatial attention via top-down modulation of sensory areas, little is known about the mechanisms underlying the generation of this bias, or how it is conveyed to, or interacts with, sensory cortex. It has been demonstrated that neuronal gamma band synchronization is significantly increased in cortex activated by the presence of a visual stimulus, and is enhanced among neurons activated by an attended stimulus. This increased synchronization has been shown to occur both within and between cortical areas. Gamma frequency activity thus appears to be an attentionally modulated correlate of stimulus drive, and is a likely target of top-down modulation. Beta frequency processes appear to play a converse role as they have been increasingly linked to endogenous cognitive mechanisms, such as selective attention - suggesting that beta frequency oscillations may mediate top-down control. Based on these considerations, we hypothesized that top-down beta frequency influences emanating from the fronto-parietal system modulate stimulus driven bottom-up gamma band synchronization between visual areas via a cross-frequency interaction.
We recorded local field potentials with a 252 channel electrocorticographic grid in two rhesus macaques performing a cued covert spatial attention task. We demonstrate that top-down beta frequency interactions, indexed by Granger causality (GC), between posterior parietal area 7a and area V1, are enhanced with spatial attention to the corresponding visual hemifield, thus providing a suitable neural substrate for top-down bias. Crucially, we found a positive trial-by-trial correlation between top-down beta GC, between posterior parietal area 7a and area V1, and bottom-up gamma GC, between areas V1 and V4. This suggests that top-down bias modulates stimulus processing via a cross-frequency linkage between top-down beta frequency oscillations, and bottom-up stimulus driven gamma oscillations. It is possible that the coupling of beta and gamma rhythms within a unit of cortex, such as a macrocolumn, comprise a general computational circuit for top-down modulation whereby gamma frequency ascending stimulus drive may be modulated by descending beta frequency gain control.

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Poster

261. **Spatial Attention**

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Topic: D.04. Vision

Support: CIHR

NSERC

Title: The influence of irrelevant distracters on the detection sensitivity of area MT to small changes in motion direction

Authors: *P. S. KHAYAT, J. MARTINEZ-TRUJILLO;
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Abstract: Most neurons in macaque area MT can reliably encode the direction and speed of a moving stimulus through fluctuations in their firing rates. Additionally the neuron's sensitivity to motion attributes correlates with the animal's performance during motion detection and discrimination tasks. These effects have been typically observed when an single moving stimulus was presented inside an MT neuron receptive field (RF), and distracter stimuli were either absent, or located outside the RF. How the ability of MT neurons to detect changes in motion attributes of a target inside their RFs varies in the presence of distracters located nearby remains poorly investigated. Moreover, whether and how the attributes of distracters (i.e., contrast and motion direction) modulate the neurons detection performance is unknown. Here, we investigated whether and how the contrast of a task irrelevant distracter inside MT neurons RF influences the detection of a motion direction change in a target. Monkeys were trained to detect a brief motion direction change in a target RDP, in the presence of another distracter RDP, both located inside the RF. The target RDP moved in the neuron's null direction, while the distracter moved in the preferred direction but had across trials different contrast levels. We examined the firing rates and the neural detection sensitivity (aROC) to the direction change of 73 cells, as a function of the distracter contrast. Although the target direction change was always the same, regardless of the distracter contrast, we found that neural activity reliably signaled the target event only when the distracter's contrast was low (aROC > 0.58). The strength of this effect was correlated with the animal's detection performance. Interestingly, across neurons the degree of distracter interference, measured by the neural detection sensitivity and the animal's behavioral performance, appeared to depend on the strength of response normalization. These results show that distracter attributes modulate the ability of MT neurons to detect changes in the direction of moving stimuli and suggest that response normalization may be one mechanism by which this effect can be explained.

Disclosures: P.S. Khayat: None. **J. Martinez-Trujillo:** None.

Poster

261. **Spatial Attention**

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Program#/Poster#: 261.04/PP1

Topic: D.04. Vision

Support: BMBF, Grant number 01GQ1005C

Title: Spatial attention enhances the saliency of changes in motion direction at the expense of an accurate representation in area MT

Authors: *V. MEHRPOUR[1], J. MARTINEZ-TRUJILLO[2], S. TREUE[1,3];
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Abstract: The medial temporal area in the visual cortex (MT) plays a central role in the processing of visual motion information in primates. The activity of MT neurons has been shown to precisely encode the direction of visual motion and to be directly linked to accurate motion perception.
The response of MT neurons is enhanced by the allocation of spatial attention to their receptive field (RF). This attentional modulation has been typically investigated with a paradigm where the animal is trained to detect a direction or speed change in one of several moving visual stimuli located inside and outside the neurons' RF. Single neuron responses are then compared between different attentional conditions in the steady state period before the motion change. Much less is known about the neural responses around the transient change and their attentional modulation.
Visual responses of 52 MT neurons were recorded from two hemispheres of two awake, behaving rhesus monkeys performing a motion direction change detection task: while the animal kept its gaze on a central fixation point, a static random dot pattern (RDP) was briefly shown either inside or outside the receptive field, cueing the animal as to the target's location in the upcoming trial. Subsequently, two moving RDPs were simultaneously presented inside and outside the RF. At a random time the target or distracter direction changed by 25° for 200ms. The animal's task was to indicate a target change by releasing a lever and ignoring changes of the distracter.
Using 12 equally spaced directions we estimated the MT population response before and during the direction change. As expected, the profile accurately represented the stimulus direction before the change, with an enhanced response in those trials where the stimulus inside the RF was the target. However, after a change of the stimulus in the RF, the response profile indicated a direction change larger than the physical change. For distractor stimuli this error was approx. 8° and 13° for target stimuli.
Our data demonstrate that the neural encoding of transient motion direction changes does not match the physical direction change. Rather, the population of MT neurons exaggerates the magnitude of a direction change, making it more salient. Furthermore, the magnitude of the

encoded direction change is almost twice as high for attended vs. unattended stimuli. This finding suggests that the role of attention is not only to increase the strength and accuracy of an attended stimulus' representation but also to enhance stimulus changes, in particular if they are behaviorally relevant (such as a direction change in our experiment).
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Disclosures: V. Mehrpour: None. **J. Martinez-Trujillo:** None. **S. Treue:** None.

Poster

261. **Spatial Attention**

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Program#/Poster#: 261.05/PP2

Topic: D.04. Vision

Support: NIH Grant EY018683

Title: Attentional modulation of LGN-V1 connectivity in the macaque monkey

Authors: *V. L. MOCK[1], A. M. BASTOS[2,3], J. R. HEMBROOK-SHORT[1], J. M. HASSE[1], F. BRIGGS[1];
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Abstract: Increasing evidence suggests that attention modulates the activity of specific neuronal populations within a brain area and influences how information is communicated from one neuronal population to another. Parallel lines of research suggest that different neuronal populations, separated into laminar compartments, synchronize their activity at specific frequencies measured in the local field potential (LFP). We are examining how attention alters specific frequency modulations in LFPs recorded across the layers of primary visual cortex (V1) and/or frequency coupling between the visual thalamus (lateral geniculate nucleus or LGN) and V1. We record LFPs through electrodes placed within retinotopically-aligned regions of the LGN and V1 in awake-behaving monkeys performing a contrast change detection task that requires covert shifts in visual spatial attention. We assess the laminar location of V1 recording sites based on frequency analysis of recorded LFPs and current-source density analysis of linear contacts spanning the V1 cortical depth. Our preliminary results suggest that LGN/V1 recordings demonstrate three basic frequency profiles in response to visual stimulation: 1) enhanced gamma-frequency responses at V1 sites, attributed to superficial layers; 2) enhanced beta-frequency responses at LGN sites with enhanced low-frequency activity at V1 sites, attributed to

granular layers; and 3) enhanced alpha-frequency responses at V1 sites, attributed to deep layers. Interestingly, we observe attentional enhancement of coherence between LGN and V1 sites at the frequencies that show enhanced power according to laminar location. Furthermore, analysis of directed interactions using Granger causality reveals attentional modulation of directional connectivity between LGN and V1 in the feedforward direction for beta-frequencies. Together, these results support the notion that attention selectively modulates the activity of specific neuronal populations within a cortical area and that this specificity influences how information is communicated in the early visual pathways.

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Poster

261. **Spatial Attention**

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Topic: D.04. Vision

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NIH Grant EY018683

Title: Attentional modulation of distinct neuronal populations in primate V1

Authors: *J. R. HEMBROOK-SHORT, V. L. MOCK, J. M. HASSE, F. BRIGGS; Physiol. and Neurobio., Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: Increasing evidence suggests that attention differentially modulates the activity of specific neuronal populations within a certain brain area. However, exactly how attention differentially alters neuronal activity in specific populations or laminar compartments is not known. In order to address whether and how attention differentially modulates the activity of specific neuronal populations, we examine attentional modulation of identified neuronal cell types at known laminar locations within primary visual cortex (V1) of awake behaving monkeys. A 24-contact linear multi-electrode array is used to record single-unit, multi-unit, and local field potentials (LFPs) within the parafoveal region of V1 in monkeys performing a contrast change detection task that requires covert shifts in visual spatial attention. The laminar location of V1 recording sites is assessed based on spectral analyses of recorded LFPs and current source density analyses of linear contacts spanning the V1 cortical depth. Also, downstream distance (in time and synaptic delay) of V1 recorded units is determined relative to visual thalamic activity

recorded through separate electrodes chronically implanted within the visual thalamus. The visual physiology of recorded single-units is determined based on their responses to drifting sinusoidal gratings that vary in contrast, orientation, size and spatial and temporal frequency. Finally, attentional modulation of neuronal activity is assessed by comparing responses on trials in which animals attend toward versus away from visual stimuli that overlap recorded receptive fields. Our preliminary data suggest that attentional modulation of neuronal firing rate varies according to laminar location and neuronal cell type. These results will greatly increase our understanding of how attentional signals are communicated among neuronal populations.

Disclosures: J.R. Hembrook-Short: None. **V.L. Mock:** None. **J.M. Hasse:** None. **F. Briggs:** None.

Poster

261. **Spatial Attention**

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Topic: D.04. Vision

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NS059753

Title: Increased attentional suppression of cortical LFP with practice time

Authors: *X. LI[1], R. LASHGARI[1], M. JANSEN[1], Y. BERESHPOLOVA[2], H. A. SWADLOW[2,1], J.-M. ALONSO[1,2];
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Abstract: Spatial attention is known to modulate the activity of neurons in primary visual cortex, however, it remains unclear how these modulations are affected by extended periods of practice. To measure the time course of attentional modulations, we chronically implanted an array of ultra-thin sharp electrodes to record local field potentials (LFPs) in area V1 (Swadlow et al., 2005). Two monkeys were trained to perform two attentional tasks: orientation discrimination and color detection. In both tasks, the monkeys covertly attended a drifting grating that was cued at the beginning of the trial and that could be either inside or outside the LFP receptive field. In both tasks, the LFP was reliably modulated by attention around the time preceding the orientation/color change (average: 8.5/7.3%, p=0.005/0.009) but not at the time following the onset of the cued grating (average: 2.0/1.0%, p=0.380/0.658). Spatial attention

suppressed low LFP frequencies (0.5 - 13 Hz) and enhanced high gamma frequencies (70 - 160 Hz) in both tasks and both monkeys. The low-frequency suppression averaged -9% to -16% in the orientation task (delta: -16.4%; alpha: -10.5%; theta: -9.3%, $p<0.001$, Wilcoxon tests) and -8% to -10% in the color task (delta: -8.2%; alpha: -9.9%; theta: -10.8%; $p<0.001$, Wilcoxon tests). The high-gamma frequency enhancement was more modest and averaged +3.9% in the orientation task ($p<0.001$, Wilcoxon test) and +2% in the color task ($p=0.04$, Wilcoxon test). Interestingly, the magnitude of the attentional modulation was significantly correlated with practice time in both monkeys, but only for the orientation task. In the orientation task, practice time (monkey R: 20 months, monkey S: 6 months) was negatively correlated with the magnitude of the attentional modulations at low LFP frequencies and positively correlated at high LFP frequencies. The negative correlations (increased low-frequency-suppression with time) ranged from -0.7 to -0.8 in monkey S (delta: $r=-0.69$, $p=0.026$; alpha: $r=-0.85$, $p=0.002$; theta: $r=-0.76$, $p=0.009$) and -0.6 to -0.8 in monkey R (delta: $r=-0.63$, $p=0.050$; alpha: $r=-0.80$, $p=0.005$; theta: $r=-0.75$, $p=0.012$). The positive correlations (increased high-frequency-enhancement with time) were +0.71 ($p=0.021$) for monkey S and +0.76 ($p=0.010$) for monkey R. In the color task, attentional modulations did not change with practice time (monkey R: 12 months, monkey S: 6 months) with exception of a reduction in alpha suppression for monkey S ($r=+0.63$, $p=0.050$). These results demonstrate that the cortical effect of spatial attention changes over time, as it would be expected if focused attention plays an important role in perceptual learning.

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Poster

261. **Spatial Attention**

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Title: Predictive power of area V4 local field potentials in determining the state of attention

Authors: *J. MAYO[1], O. GOZEL[2], J. H. R. MAUNSELL[1];
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Abstract: Everyday behaviors require flexible mechanisms for processing visual information. The allocation of attention to specific regions of visual space is one such way in which the brain prioritizes information related to salient stimuli. Most work on the neuronal correlates of visual attention has focused on sustained attention to specific sites in the visual field. However, our focus of attention can change from one location to another frequently and rapidly. Methodological constraints largely prevented the investigation of these changes in attention at behaviorally relevant timescales. Thus, much less is known about how and when changes in attentional state occur at the level of single neurons.
Recent work in our laboratory measured the simultaneous spiking activity of small populations of neurons in visual area V4 to produce trial-by-trial measures of visual attention (Cohen and Maunsell, 2010). Here, we extend this work by examining the predictive power of local field potentials (LFPs) in a similar task. Two monkeys were each implanted with bilateral, 48-electrode arrays in V4 and trained in an orientation change-detection task. Two stimuli were presented simultaneously on a video display, with each stimulus located in the overlapping receptive fields of each electrode array. Stimuli were odd-symmetric Gabors at 100% contrast, sinusoidally counterphased at 10 Hz. Monkeys were trained to fixate in the center of the display and saccade to a stimulus only when it changed orientation. Attention to a stimulus was cued by instruction trials containing a salient cue stimulus at the beginning of each block of trials. Stimulus changes in a block occurred at the cued location ("valid") on 80% of the trials and at the other location ("invalid") for 20% of the trials. Cued locations alternated between blocks. We tested a range of task difficulties (i.e., orientation changes) and measured behavioral performance, spiking activity, and LFPs in over 75 recording sessions.
We found that LFPs sampled for 250 ms before the stimulus change--a putative measure of the animal's attentional state--could be used to predict with relatively high accuracy whether the animals were going to detect or miss the impending stimulus change in that trial. LFPs in the gamma range (30-80 Hz) had predictive power comparable to that of spiking activity. These results suggest that the combination of spiking activity and LFPs can be a powerful predictor of the behavioral outcome of the trials, useful for temporally precise studies of changes in the state of attention.

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Poster

261. **Spatial Attention**

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The Hundred Talent Program, Chinese Academy of Sciences

Title: Acetylcholine iontophoresed into the lateral intraparietal area enhances neuronal firing and improves the monkey's behavior on a difficult visual search task

Authors: *X. WANG[1], M. ZHANG[2], M. E. GOLDBERG[1,3];
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Abstract: Acetylcholine (Ach) is a major neuromodulator in the parietal cortex of the monkey, but little is known about its function. We previously showed that when monkeys perform interleaved difficult and easy visual search tasks, the baseline activity in LIP, measured while the monkeys fixate waiting for the search array to appear, predicts the monkey's success or failure on the difficult task, correlates directly with the intensity of the visual response to the array, the monkey's performance on the current trial, and the monkey's manual reaction time in task, and inversely with the monkey's history of success. The baseline effect is not spatially tuned, and is unrelatged to the monkey's locus of attention (Wang et al., SfN 2012). To study the cellular mechanisms, we applied ACh (0.1M), or the nicotinic receptor antagonist mecamylamine (0.1M), or the muscarinic receptor antagonist scopolamine (0.1M) iontophoretically in the LIP via a 7-barreled pipettte with tungsten wire in the central barrel with which we could measure the activity of single neurons while the monkey performed the search task. We found that Ach increases both the baseline activity and visual responses for both successful and unsuccessful trials. ACh also improves performance on the search task when the search target is in the receptive field of the neuron under study. Both mecamylamine and scopolamine suppress the baseline effects and visual responses of LIP neurons. We suggest that cholinergic neuromodulation of parietal activity is important in regulating the efficiency of behavior in the monkey, and that both nicotininc and muscarinic receptors may be involved.

Disclosures: X. Wang: None. **M. Zhang:** None. **M.E. Goldberg:** None.

Poster

261. **Spatial Attention**

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Helen Hay Whitney Fellowship

Title: Decoupled neural and behavioral correlates of attention in a dual orientation discrimination task

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Abstract: A large body of evidence links the behavioral hallmarks of attention (increased performance and decreased reaction time) with neuronal response gain in sensory areas like V4. The import of other cognitive factors, like reward and motivation, on response gain have been difficult to disentangle from those of spatial attention. Here, in an effort to understand the range of cognitive influences on visual perception, we systematically vary from trial-to-trial the reward available in each of 2 locations, decoupling effects of total expected reward and expected reward in a given location from those of spatial asymmetries in reward.
We recorded single unit activity in V4 while monkeys performed a "reward-cued' orientation discrimination task in which each trial has two phases. First, two motivational cues appear indicating the reward value (large or small) associated with correct performance in each hemifield. Second, two oriented stimulus patches appear in opposite hemifields. The monkey must discriminate the orientation of one of the stimulus patches, which is randomly selected by the experimenter and only identified to the monkey after the stimulus patches have been extinguished. Therefore, preceding motivational cues determine the behavioral relevance of each stimulus patch. Motivational cues define four reward scenarios: large reward in receptive field, small opposite (LS); small reward in receptive field, large opposite (SL); both targets largely rewarded (LL); and both lowly rewarded (SS).
We find that when one location is associated with a high reward, and the other with low reward (ie LS or SL), performance is increased and reaction time is decreased at the high reward location. In keeping with previous findings on spatial attention, these behavioral hallmarks of attention are associated with increased firing rates in V4 neurons. At low reward locations, average performance is markedly higher on SS trials than SL trials. Surprisingly, this behavioral difference occurs without commensurate increases in neuronal response gain. Conversely, comparing LL to SS trials (where the absolute values of both targets change, but relative values

of targets do not), we find substantial neuronal response gain in the LL condition that is not accompanied by commensurate behavioral benefits. Taken together, these data suggest that response gain may primarily affect attentional behavior by promoting efficient target selection.

Disclosures: J.K. Baruni: None. **B. Lau:** None. **C.D. Salzman:** None.

Poster

261. **Spatial Attention**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 261.11/PP8

Topic: D.04. Vision

Support: NIH RO1-MH092345

James S. McDonnell Foundation grant

NIH RO1-MH068004

Title: Manipulating attention strategy alters patterns of neural gain in human cortex

Authors: *S. ITTHIPURIPAT[1], J. O. GARCIA[2], N. RUNGRATSAMEETAWEEMANA[3,4], T. C. SPRAGUE[1], J. T. SERENCES[1,2];
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Abstract: Over the last several decades, spatial attention has been shown to influence the activity of neurons in visual cortex in a number of different ways. In turn, these conflicting observations have inspired competing models to account for the influence of attention on perception and behavior. One recent model, the normalization model of attention (NMA), attempts to reconcile previous findings by showing how different modulatory patterns can arise due to differences in the spatial extent of attention. Here, we directly tested this model using electroencephalography (EEG) to assess steady-state visual evoked potentials (SSVEPs) in human subjects. While fixing the physical properties of the stimulus display, we show that highly focused spatial attention primarily enhances neural responses to high-contrast stimuli (response gain), whereas distributed attention primarily enhances responses to medium-contrast stimuli in a manner similar to increasing the physical salience of the stimulus (contrast gain). Moreover, we found that the scope of attention modulated responses evoked by both attended and ignored stimuli. Together, these data suggest that different patterns of neural modulation do not reflect fundamentally different neural mechanisms, but instead reflect changes in spatial extent of

attention as implemented by different attentional strategies.

Disclosures: S. Itthipuripat: None. **J.O. Garcia:** None. **T.C. Sprague:** None. **J.T. Serences:** None. **N. Rungratsameetaweemana:** None.

Poster

261. **Spatial Attention**

Location: Halls B-H

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Topic: D.04. Vision

Support: NIH Grant MH092345

Title: Near real-time spatial reconstructions of visual stimuli with EEG: Exploring the dynamics of spatial attention

Authors: *J. O. GARCIA[1], K. E. KAYE[1], T. C. SPRAGUE[2], J. T. SERENCES[2];
[1]Psychology, [2]Neurosciences, UC San Diego, La Jolla, CA

Abstract: Recently, patterns of fMRI responses across areas of visual cortex have been used to label, or to decode, the precise orientations (Kamitani & Tong, 2005), objects (Haxby et al., 2001), and even natural image (Kay et al., 2008) that a subject is viewing. Since then, encoding analyses have been developed, where, instead of using neural activity to predict information about a particular stimulus, a model of neural encoding is used to reconstruct a representation of a stimulus based solely on patterns of brain activity (Naselaris et al., 2011; Serences & Saproo, 2012; Tong and Pratte, 2012). We have recently demonstrated that encoding models can also be used to extract high temporal resolution information about stimulus orientation based on the spatiotemporal pattern of EEG responses across the scalp (Garcia et al., 2013). Here, we extend this technique to map the near real-time deployment of spatial attention to different positions within the visual field. We use high density EEG to measure steady state visual evoked potentials (SSVEPs) to small contrast-reversing (20Hz) discs in 36 locations arrayed across the visual field. Subjects were instructed to discriminate targets that appeared within the small discs or to report fixation brightness changes. We show (1) multivariate encoding algorithms can exploit the scalp distribution of SSVEP power to reconstruct an accurate representation of the visual stimulus, (2) these representations are influenced by intrinsic oscillatory activity, and (3) are modulated by attention.

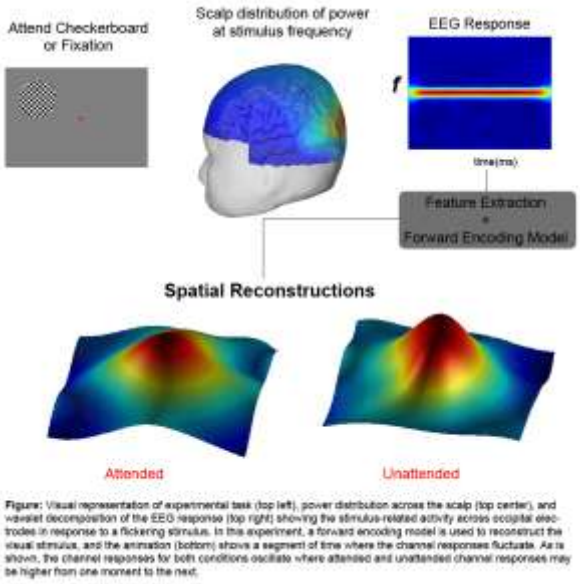

Figure: Visual representation of experimental task (top left), power distribution across the scalp (top center), and wavelet decomposition of the EEG response (top right) showing the stimulus-related activity across occipital electrodes in response to a flickering stimulus. In this experiment, a forward encoding model is used to reconstruct the visual stimulus, and the animation (bottom) shows a segment of time where the channel responses fluctuate. As is shown, the channel responses for both conditions oscillate where attended and unattended channel responses may be higher from one moment to the next.

Disclosures: J.O. Garcia: None. **K.E. Kaye:** None. **T.C. Sprague:** None. **J.T. Serences:** None.

Poster

261. **Spatial Attention**

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Title: The spreading of attentional selection within an object's visual boundaries is a periodic process

Authors: *I. C. FIEBELKORN, Y. B. SAALMANN, S. KASTNER;
Princeton Univ., Princeton, NJ

Abstract: Much of the recent work investigating attentional selection has focused on its temporal dynamics, with several studies linking the behavioral consequences of attention to underlying oscillatory phenomena (e.g., phase reset). In the present study, we probed human behavior to investigate the temporal dynamics of visual-target detection under two conditions of attentional selection: spatial and object-based selection. Previous research has shown that preferential processing spreads from a cued location to uncued locations, if those locations are part of the same object (i.e., encompassed by the same visual boundaries). What remains unclear, however, is the extent to which attentional selection at the uncued, same-object location (i.e., object-based selection) shares common underlying neural processes with attentional selection at the cued location (i.e., spatial selection). Does attentional selection at these two locations reflect (1) a single set of neural processes that is dynamically guided by both location and object properties, or rather, (2) separable neural processes? Here, we varied the cue-target interval (300-1100 ms) and examined how detection of a near-threshold visual target evolves under conditions of spatial and object-based selection. We used an uncued location on a second object to track detection in the absence of spatial and object-based selection. Our data reveal an inverse relationship between spatial and object-based selection, which is attributable to antiphase coherence (at ~4 Hz) between visual-target detection at the uncued same- and different-object locations. That is, periodic enhancement of visual-target detection under conditions of object-based selection is accompanied by a periodic decrement of visual-target detection at the different-object (baseline) location. A comparison between visual-target detection under conditions of spatial and object-based selection, on the other hand, revealed periodicity in the theta range (at ~8 Hz) with significant cross-condition coherence and a near-zero phase shift. Such periodicity in the theta range was not present at the different-object (baseline) location. These results thus suggest the following: (1) the spreading of attentional selection within an object's visual boundaries is periodic rather than sustained, alternating between periods when the same-object location is preferentially processed and periods when the same- and different-object locations are equivalent, and (2) visual-target detection under conditions of spatial and object-based selection has a similar spectral architecture, supporting the case for common underlying neural processes.

Disclosures: I.C. Fiebelkorn: None. **Y.B. Saalmann:** None. **S. Kastner:** None.

Poster

261. **Spatial Attention**

Location: Halls B-H

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Topic: D.04. Vision

Support: NSF BCS – 1228595

Title: Entrainment of neuronal oscillations as a flexible mechanism controlling visuospatial attention

Authors: *M. J. GRAY, H.-P. FREY, J. J. FOXE;
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Abstract: A long line of research has demonstrated that oscillatory coupling between groups of neurons can act as an efficient means for encoding and selecting information. Building on this concept, a recent influential hypothesis has proposed that the entrainment of low-frequency neuronal oscillations to a stimulus serves as a fundamental mechanism for perceptual selection within a complex and dynamic environment (Lakatos, 2009). EEG and ECoG experiments in humans have since demonstrated low-frequency entrainment to rhythmically-presented auditory and visual stimuli, and that modulation of the phase of the entrained oscillations can operate to select between these modalities. However, such experiments have used only one stimulus stream and one entrainment frequency, and thus it remains to be seen to what extent this mechanism can be used to select between multiple stimuli within the same modality. To answer this question, we devised a simple selective attention task in which subjects were required to covertly sustain their attention to one of two simultaneously presented checkerboards, each rhythmically alternating at a distinct frequency (1.3 Hz and 1.5 Hz). Using high-density EEG and leveraging long recording epochs to obtain a very high frequency resolution, we show that endogenous oscillatory entrainment can operate to select between multiple temporally and spatially distinct visual stimuli. Analysis of inter-trial phase coherence (ITPC) revealed selective and highly significant entrainment at the frequency of the attended stimulus. Significant ITPC was also seen at numerous harmonics of the fundamental stimulus frequency, extending up to around 8 Hz, suggesting that attentional selection and monitoring recruits oscillations well outside of the delta range, extending the original proposal. Furthermore, the topographical distribution of this broad range of specific entrainment frequencies was largely bilateral and extending into parietal areas, supporting the notion that synchronous oscillations provide an efficient means for transmitting relevant stimulus information from early sensory cortex for higher-order processing. Our findings provide evidence that the entrainment of a distributed, multi-frequency network of neuronal oscillations acts as a flexible means for the selection of temporal regularities unique to an attended stimulus within a given sensory modality.

Disclosures: M.J. Gray: None. **H. Frey:** None. **J.J. Foxe:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

Support: JST PRESTO

Title: Characteristics of trial-to-trial spike count variability in MT neurons are consistent with bottom-up components of decision related response modulation

Authors: *H. KUMANO, T. UKA;
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Abstract: Decision related response modulation (choice probability: CP) is observed in many sensory cortex and is believed to represent the association between perceptual choice and neuronal activity. Possible sources for the association include bottom-up and top-down components. A bottom-up explanation states that fluctuations in common noise among sensory neurons influence perceptual decision, whereas a top-down explanation states that signals of central origin such as feature attention influence the activities of sensory neurons. It has, however, been difficult to distinguish between these possibilities.
Here we report a new method for distinguishing between a bottom-up and top-down account of CP. We first predicted the amount of trial-to-trial spike count variability based on bottom-up and top-down models. In the bottom-up model, simulated sensory neurons fired with a spike count variability that was equal to the mean. Decisions were made via a circuit consisting of multiple independently firing neurons. On each simulated trial, responses of a simulated sensory neurons and the decision outcome was determined, and CP was calculated across trials. In this case, the overall variability in response to a visual stimulus did not change depending on CP, but variability measured separately for each behavioral choice decreased with higher CP. In the top-down model, a decision dependent signal was added to the neuron's response. In this case, variability measured separately for each behavioral choice did not depend on CP, but the overall variability increased with higher CP.
To determine which model better described the data, we analyzed CP measured in area MT during a task switching paradigm, where two tasks (direction discrimination and depth discrimination) were randomly interleaved (Sasaki and Uka, 2009, Neuron). For this dataset, CPs of MT neurons can be different depending on task, particularly for incongruent neurons whose preferred direction and preferred depth were related to opposite choices in the two tasks. Measurement of CP in two tasks thus allowed us to analyze the relative change in neuronal variability independent of the large fluctuations across individual neurons. The overall variability in response to visual stimulus did not change depending on CP. Conversely, variability measured separately for each behavioral choice decreased with higher CP, in line with the prediction of the bottom-up model. Overall, our results are consistent with the bottom-up account of CP.

Disclosures: H. Kumano: None. **T. Uka:** None.

Poster

262. **Visual Decision Making**

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Topic: D.04. Vision

Support: NIH Grant DC010450

NIH Grant EY019087

Title: Ventral intraparietal (VIP) inactivation does not affect mulitsensory heading perception

Authors: *E. M. KLIER[1], S. LIU[2], Y. GU[3], G. C. DEANGELIS[4], D. E. ANGELAKI[2];
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Neurosci., Baylor Col. Med., Houston, TX;
[3]Inst. of Neurosci., Chinese Acad. of Sci., Shanghai, China; [4]Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: While deficits in sensory discrimination after reversible chemical inactivation are well documented for early sensory (e.g., middle temporal area) and late motor (e.g., frontal eye fields) cortical areas, its affects on intermediary sensorimotor areas are not well characterized. The ventral intraparietal area (VIP), like the medial superior temporal area (MST), shows strong multisensory convergence, including optic flow and vestibular signals, and strong correlations between neural activity and perceptual choice (i.e., choice probabilities) during heading discrimination tasks. To test for causal deficits in heading perception, muscimol (a GABA agonist) was injected bilaterally into area VIP of two macaque monkeys. Cues for the direction of motion were given by either visual motion via optic flow, vestibular motion via a motion platform, or combined visual and vestibular stimuli via synchronized optic flow and platform motion. This experiment was conducted under nearly identical conditions as a similar inactivation study in MSTd (Gu et al., J Neurosci, 2012). Across both animals, VIP heading thresholds did not increase significantly after inactivation (visual = 2.50 ±1.56; vestibular = 2.03 ±0.81; combined = 1.50 ±0.66) from their baseline levels (visual = 2.76 ±1.70; vestibular = 1.75 ±0.67; combined = 1.59 ±0.66) (t-tests, visual p = 0.55; vestibular p = 0.10; combined p = 0.88). In contrast, MSTd inactivation showed significant increases in heading thresholds across all stimulus conditions (Gu et al., J Neurosci., 2012). Notably, there is a clear dissociation between effects of inactivation in VIP and MSTd and choice probability measurements. Whereas inactivation disrupted heading perception in MSTd but not VIP, choice probabilities were much stronger in VIP than MSTd during the same task (Chen et al., J Neurosci, 2013). This indicates

that choice probabilities may not always be predictive of a causal contribution of neurons to perception. Taken together, these results suggest that sensorimotor parietal areas may not directly affect perception after chemical inactivation. This may be due to the fact that their functions are distributed across several cortical areas, or that their responses correlate with the outcome of a decision process without directly contributing to that process.

Disclosures: E.M. Klier: None. **S. Liu:** None. **Y. Gu:** None. **G.C. DeAngelis:** None. **D.E. Angelaki:** None.

Poster

262. **Visual Decision Making**

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Topic: D.04. Vision

Support: University Research Foundation, University of Pennsylvania

PENN Medicine Neuroscience Center Pilot Grant

NIH Grant EY022411

Title: Reward asymmetry modulates perceptual decision-related activity in the monkey prefrontal cortex

Authors: *L. DING;
Dept of Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Perceptual decisions about the presence or identity of external sensory inputs can be influenced by non-sensory, internal factors such as reward expectation. Prefrontal cortical neurons encode decision-related signals for both perceptual and value-based decision making, making them likely candidates to mediate modulation of perceptual decisions by reward expectation. A recent, preliminary study demonstrated reward-modulated activity in the prefrontal cortex in monkeys performing an asymmetric-reward visual motion speed-discrimination task. However, it remains unclear how such reward modulation relates computationally to the reward-biased behavioral reports.
Behavioral performance on a random-dot, visual motion direction-discrimination ("dots") task is well described by accumulate-to-bound computational frameworks. Previously we and others have shown that, on the dots task, frontal eye field (FEF) activity represents key features of the accumulate-to-bound process, and asymmetric choice-reward associations can induce a

behavioral reward bias that can be approximated by an adjustment in the starting value of the motion-accumulation process. In this study, I examined how asymmetric choice-reward associations modulate single-neuron activity in the FEF and how such modulation relates computationally to reward biases measured behaviorally.
Single FEF neurons were recorded in a monkey performing an asymmetric-reward reaction-time dots task. In alternated blocks, the choice associated with a FEF neuron's response field (RF) was paired with a larger or smaller reward than the other choice. A combination of statistical tests and validation by visual inspection was used to identify neurons with choice-selective activity and examine how motion strength and reward expectation influence such neurons. I focused on a preliminary sample of 23 cells with choice-selective activity before saccade onset, i.e., those likely contributing to impending choices, and observed three types of reward modulation: 1) before motion stimulus onset, 5 cells had increased baseline activity when the larger reward was paired with RF choices; 2) during motion viewing before RF choices, 2 cells had build-up activity that was modulated by both motion strength and expected reward size; and 3) just before RF-choice saccades, 11 cells had activity levels that were modulated by a combination of expected reward size and motion strength, instead of fixed final levels common to all conditions. These results suggest that reward asymmetry largely influences the starting value and bound height of the accumulate-to-bound-like process in the prefrontal cortex.

Disclosures: L. Ding: None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

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Topic: D.04. Vision

Support: NIH Grant EY019273

The McKnight Foundation

Title: The representation of object similarity but not choice certainty in LIP

Authors: *W. S. ONG[1], J. W. BISLEY[2];
[1]Dept. of Neurobio., Duke Univ., Durham, NC; [2]UCLA, Los Angeles, CA

Abstract: We are able to search for and locate specific objects in our environment by looking for its features and mentally comparing it to the object we are looking for. As such, while we are searching for that specific object, our attention may be drawn more to an object that is similar to

the target compared to an object that is different from the target. To do this, we would need to integrate both the features and location of objects in our visual space and compare them with the target object. We propose that the lateral intraparietal area (LIP) is key to this process as it receives direct input from the ventral, and dorsal visual processing streams as well as being part of the parietal-frontal network. Here, we investigate whether the activity across LIP combines bottom-up percepts with top-down instructions to create a map based on the similarity of the objects.

Two animals were trained to perform a 2-alternative forced choice, match-to-sample task. Each day, 5 line objects which varied parametrically from each other were randomly chosen from a set of 243. In this task, the sample object is presented at the fixation point at the center of the screen. The 2 choice objects are presented on either side of the fixation point, one of them in the receptive field of the neuron being recorded from. The choice objects can be presented up to 600 ms before or 200 ms after the presentation of the sample object. After the fixation point is extinguished, the animal makes a saccade to the object in the periphery that matches the sample object for a juice reward.

We recorded the responses from 204 LIP neurons in 2 animals. Using the performance of the animals across the session as a proxy for similarity, we show that the neural response to the non-target object in the receptive field of the neuron varies as a function of perceived similarity as early as 250 ms from the onset of the sample object. This average correlation increases in strength up to the time of the saccade onset. The monkey maintains fixation during this time as dictated by the task, and on average, the saccade occurs 850 ms after the sample onset. This correlation is not tied to the certainty or reward probability of the stimulus in the receptive field, as we do not find any significant correlation between the animal's performance and the neural response to the target object in the receptive field.

We suggest that the activity of LIP neurons represents the perceived similarity of the objects the animal is comparing with the sample object, and that the activity in LIP does not represent certainty or purely all-or-nothing motor movements.

Disclosures: W.S. Ong: None. **J.W. Bisley:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

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Program#/Poster#: 262.05/PP16

Topic: D.04. Vision

Support: JST PRESTO

Title: Dynamics of sensory information accumulation in LIP during task switching

Authors: *Y. SUDA, H. KUMANO, T. UKA;
Dept. of Neurophysiol., Juntendo Univ. Grad. Sch. of Med., Tokyo, Japan

Abstract: Switching behavior based on multiple rules is a fundamental ability for humans to behave properly according to context. Although the neural activity involved in the accumulation process for perceptual decision making has been well studied in the lateral intraparietal area (LIP) of the macaque, little is known about how the accumulation process changes when monkeys are required to read out distinct information from identical stimuli depending on task. To elucidate this question, we examined neural activity in LIP while monkeys performed a reaction time task version of a task switching paradigm. A Japanese macaque was trained to switch between direction and depth discrimination tasks. On a given trial, the color of the fixation point indicated whether the monkey had to discriminate motion direction (UP or Down) or stereoscopic depth (FAR or NEAR). The monkey was allowed to make a saccadic eye movement as soon as a decision was made. Difficulty of the tasks was varied by changing the percentage of coherently moving and binocularly correlated dots in the visual stimulus. While the monkey performed task switching, we recorded extracellular activity from isolated neurons in LIP. Using a memory-guided saccade task, we focused on neurons that showed delay activity. Switching performance was evaluated by calculating a switch ratio (SR). Mean SR for the direction discrimination task was 0.81 and that for depth discrimination task was 0.76, suggesting that the monkey successfully switched between the two tasks. Reaction time showed an inverted U shape as a function of the strength of the relevant stimulus feature, but was shifted horizontally depending on the strength of the irrelevant stimulus feature. Individual LIP neurons showed build-up activity depending on both motion coherence and binocular correlation, whereas their activities were similar around saccade time irrespective of these two stimulus features. Finally, the slope of the build-up activity changed depending on task so that build-up was prominent only for the stimulus dimension relevant to the task. Our results suggest that LIP neurons integrate relevant information depending on context to decide flexibly where to move the eye.

Disclosures: Y. Suda: None. **H. Kumano:** None. **T. Uka:** None.

Poster

262. **Visual Decision Making**

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MEXT Grant TO22120507

Title: Target facilitation and distractor suppression in the activity of macaque lateral intraparietal neurons during visual search

Authors: *S. NISHIDA, T. TANAKA, T. OGAWA;
Dept. of Integrative Brain Science, Grad. Sch. of Med., Kyoto Univ., Kyoto, Japan

Abstract: During visual search, neurons in the lateral intraparietal area (LIP) discriminate the target from distractors by exhibiting stronger activation when the target appears within the receptive field than when it appears outside the receptive field. It is generally thought that such target-discriminative activity is comprised of target-related facilitation and distractor-related suppression. However, little is known about how the facilitative and suppressive modulations emerge in target-discriminative activity of LIP neurons. To address this issue, we recorded activity from LIP of monkeys performing a visual search task that consisted of target-present and target-absent trials. Monkeys had to make a saccade to a target in the target-present trials, whereas they had to maintain fixation in the target-absent trials, in which only distractors were presented. By introducing the activity from the latter trials as neutral activity, we were able to separate the target-discriminative activity into target-related elevation and distractor-related reduction components. We found that most LIP neurons discriminated the target by the combination of target-related elevating and distractor-related reducing modulations or only elevating modulations in their activity, whereas very few neurons discriminated the target only by distractor-related reducing modulations. We also found that on average, target-related elevation was stronger and occurred earlier compared with distractor-related reduction. Further, as expected in previous model studies, the observed response properties were consistent with the simulation results derived from a network model with lateral inhibitory interactions. These findings provide insight into how neuronal modulation shapes target-discriminative activity during visual search.

Disclosures: S. Nishida: None. **T. Tanaka:** None. **T. Ogawa:** None.

Poster

262. **Visual Decision Making**

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Topic: D.04. Vision

Support: RO1EY11749

P30EY01319

Title: Spatial specificity of direction selectivity in the dorsolateral prefrontal cortex during memory-guided direction comparison task

Authors: *P. REN, A. SIMON, P. SPINELLI, T. PASTERNAK, Professor;
Univ. of Rochester, Rochester, NY

Abstract: As we interact with our environment, the features of objects in the visual scene are not consistently present on the retina and sensory cues used to guide visual behavior are not always available. Thus, active observers are faced with a ubiquitous task of comparing sensory stimuli across time and space. When monkeys compare directions of visual motion of two stimuli presented at the fovea, S1 and S2, separated by a delay, neurons in the dorsolateral prefrontal cortex (DLPFC) show direction selective (DS) responses suggestive of their origins in area MT. In addition, responses during S2, the comparison stage of the task, are often modulated by the direction presented during S1. However, DLPFC neurons respond to motion not only at the fovea but also across the entire visual field, receiving direct bottom-up inputs from neurons in the ipsilateral MT representing contralateral stimuli and indirectly from the opposite MT representing ipsilateral stimuli. We examined whether DLPFC retains the spatial specificity in DS characteristic of its retinotopic inputs by presenting stimuli in the contralateral and ipsilateral hemifields during the direction comparison task. We found that responses to visual motion of many DLPFC neurons changed with **Location:** they were more likely to be DS and this selectivity emerged earlier when stimuli appeared in the contralateral field. Preferred directions of neurons with DS for contralateral and ipsilateral stimuli were strongly correlated, suggesting alignment of direction information arriving in DLPF from MT neurons residing in the opposite hemispheres. Finally, response modulation during S2 also depended on stimulus location, weakening when the preceding S1 appeared in the opposite hemifield, suggesting participation of retinotopically organized cortical regions in the comparison process. Our results show that representation of visual motion in DLPFC is likely to be governed by its direct and indirect connectivity with area MT. The strong correlation between direction preferences in the two hemifields points to a mechanism that may facilitate integration of motion information across the visual field.

Disclosures: P. Ren: None. **A. Simon:** None. **P. Spinelli:** None. **T. Pasternak:** None.

Poster

262. **Visual Decision Making**

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Support: European Research Council

Wellcome Trust

EPSRC

Title: Two-photon imaging of population activity in mouse visual cortex during a 2AFC task

Authors: *C. P. BURGESS[1,2], A. RANSON[1], K. D. HARRIS[3], J. F. LINDEN[2], M. CARANDINI[1];
[1]UCL Inst. of Ophthalmology, London, United Kingdom; [2]UCL Ear Inst., London, United Kingdom; [3]UCL Inst. of Neurol., London, United Kingdom

Abstract: Our understanding of the relationship between sensory encoding and perception has been greatly advanced by neuronal recordings in animals performing perceptual decision-making tasks. However, these measurements have been typically obtained as single-unit recordings in monkeys, and are thus limited in the number of cells that have been simultaneously recorded, and by an inability to track the same units over long periods. Sensory perception, on the other hand, results from the activity of large neuronal ensembles, whose activity may vary over days reflecting the learning of the task and other associations.

To study how neuronal populations encode sensory input, and how their activity relates to perceptual decisions, we developed a visual psychophysical task in head-fixed mice, and we paired it with two-photon imaging in primary visual cortex. The task is based on two-alternative forced choice (2AFC) design, which is superior to existing go/no-go designs because it is immune to variations in criterion and motivation. In the task, mice report the location of a grating stimulus by turning a wheel and receive water reward for correct responses.

Mice typically learned the task to proficiency in 2-3 weeks, and performed equally well in test chambers and under a two-photon microscope. They typically achieved upwards of 95% accuracy for the easiest stimuli, producing high-quality psychometric curves over a range of stimulus conditions. Typical contrast thresholds (to 75 % accuracy) were ~8% (for gratings of 0.1cyc/deg).

Two-photon calcium imaging allowed the simultaneous recording from large populations of cells during task performance and recording from the same cells over successive recording days. We used a viral vector (AAV2/1) to target expression of GCaMP6 to visual cortex, and found that of ~100 cells in a typical imaging frame, over 20% were significantly visually responsive ($\Delta F/Fs$ often exceeding 20%). In some experiments, a sizeable portion of the activity showed choice

probabilities beyond that expected by chance, and psychometric curves plotted contingent on neural activity revealed choice-related shifts.
We conclude that this combination of techniques provides a powerful tool to probe the activity of neuronal populations during perceptual judgements, and for unravelling the relationship between perception and decision making.

Disclosures: C.P. Burgess: None. **A. Ranson:** None. **K.D. Harris:** None. **J.F. Linden:** None. **M. Carandini:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.09/PP20

Topic: D.04. Vision

Support: European Research Council

Wellcome Trust

EPSRC

Title: Calcium dynamics in layer 1 of mouse visual cortex during sensory behavior

Authors: *A. RANSON[1], C. P. BURGESS[1], M. CARANDINI[1], K. D. HARRIS[2];
[1]UCL Inst. of Ophthalmology, Univ. Col. London, London, United Kingdom; [2]UCL Inst. of Neurol., Univ. Col. London, London, United Kingdom

Abstract: The apical tuft dendrites of many cortical pyramidal neurons arborize in layer 1 (L1), where they receive top-down synaptic input from higher-order cortical and thalamic regions. Bottom-up inputs from sensory thalamus, instead, terminate on more proximal dendrites. Coincident activation of the distal and proximal inputs may enable neurons to integrate sensory input with attentional, predictive, or motor signals.
We measured activity in L1 of primary visual cortex using 2-photon imaging of calcium signals in head-fixed mice performing a visually-guided task. Animals had to report whether a grating of variable contrast appeared on the left or right of a computer screen by rotating a wheel. Animals were water restricted, gained a water reward for correct responses, and achieved near perfect performance when contrast was highest. To measure calcium fluctuations we used the genetically encoded calcium indicator GCaMP6, delivered via an AAV vector. This indicator reports not only suprathreshold calcium signals associated with spikes but also subthreshold

calcium signals associated with synaptic inputs.
Calcium transients in L1 reflected an integration of visual input with behavioral variables. Large and reliable calcium transients were evoked by visual stimuli presented contralaterally to the imaged hemisphere. These transients increased progressively with stimulus contrast and followed faithfully the stimulus onset. However, they were not purely sensory-driven: they were larger when the animal produced a behavioral response. In fact, calcium transients could be seen even in the absence of effective visual stimulation, when stimuli were presented ipsilaterally. These transients were smaller than those evoked by visual stimuli, and their timing matched the onset of the animal's movements better than the onset of the visual stimulus.
These results suggest that L1 of primary visual cortex integrates visual signals with non-visual signals, which we hypothesize to be carried by top-down inputs.

Disclosures: A. Ranson: None. **C.P. Burgess:** None. **M. Carandini:** None. **K.D. Harris:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.10/PP21

Topic: D.04. Vision

Support: Grants- in-Aid for Scientific Research 24300146, Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT)

Title: Naturally occurring and experimentally induced choice history biases in human observers

Authors: *A. ABRAHAMYAN, J. L. GARDNER;
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Abstract: Perceptual decisions are not always guided by sensory evidence alone, but can be biased by the results of previous choices. For example, a previous successful choice may naturally prime making the same response, while an error in the past may inhibit making the same choice. We asked if humans have natural tendencies to exhibit these types of choice history biases and if naturally occurring biases could be modified by experimentally induced manipulations of trial order.
We examined naturally occurring choice history biases while humans performed a choice task in which they reported whether a visual stimulus was presented to the left or right side of fixation. Detection difficulty varied with contrast intensity (0.8%, 1%, 1.5%, 2%, 3% Michelson contrast). Stimuli were gabor patches (1 c/deg, 6 deg width) presented 12 deg to the left or right of fixation for 500 ms at a viewing distance of 50.5 cm. Subjects reported the location of the patch by

pressing a key. Auditory feedback of success or failure was provided. By applying a probabilistic choice model, we found that 6 of 9 subjects showed a natural tendency to switch between choosing left or right side when they failed on the previous trial. Successful responses on the previous trial had little or no effect on subjects' decisions.
We also examined whether choice history biases could be experimentally induced by manipulating choice history statistics. In particular, we changed the probability of alternating stimulus presentation side after a failure to 80% in one blocked condition. In another blocked condition, we repeated stimulus on the same side after a success 80% of the time. The model revealed that subjects were induced to have a choice history bias: they were more likely to stay on the same side after a success for the latter condition. There was a weaker enhanced tendency to switch after a failure for the former condition. Variance inflation factor (VIF) calculations suggested no colinearity artifacts.
We conclude that even experienced subjects have natural choice history biases in the form of switching after an error and that these biases can be modified by changing choice history statistics. If choice history biases can be manipulated by trial history statistics, it is puzzling why natural choice biases remain even for experimental designs in which such biases only serve to hurt performance. Model simulations showed that subjects' choice biases had only minor detrimental effects on psychophysical sensitivity. This suggest that when detrimental effects of natural choice history biases are modest, choice biases that may be optimal in a wider behavioral context may be retained even if they are suboptimal in the current context.

Disclosures: A. Abrahamyan: None. **J.L. Gardner:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.11/PP22

Topic: D.04. Vision

Title: Sympathetic and parasympathetic markers of visual awareness: A window into the mind-body interaction

Authors: *B. R. SHETH[1], Z. LI[2];
[1]Dept Elec, Comp Eng, [2]Dept Elec. Comp. Eng, Univ. Houston, HOUSTON, TX

Abstract: It has long been known that the brain does not exist in isolation but exists in a body and embodied cognition implies that the brain is constrained by the body. In exploring it, the mind-body dichotomy is an important beachhead. The only way to scientifically study the mind-

body interaction is to study the interaction between the brain (mind) and the autonomic nervous system (ANS), which controls the organs of the body. Here, we focus on the influence of consciousness, or visual awareness using three paradigms - visual detection (VD), visual localization (VL), and binocular rivalry (BR) - on the ANS using heart rate (HR), heart rate variability (HRV), pupil diameter, and pre-ejection period (PEP) as measures of the activations of the parasympathetic (para) and sympathetic (sym) components of the ANS.
There were three visual paradigms; each explores visual awareness from a different angle. In BR, observers (Os) viewed two stimuli (face, house) presented to different eyes and judged when each image was consciously perceived. In VD, Os viewed a near-threshold target stimulus on 50% of trials, and judged whether they detected one or not. In VL, a target of variable intensity (near-/above-threshold) was presented on left/right side of fixation on 50% trials each, and the O had to locate it. Confidence ratings were acquired. We expect changes in the para (high frequency spectral power of HRV, pupil diameter) and sym (PEP) systems due to the orienting response (OR), which is a reflexive, immediate response to environmental change, and an additional modulation dependent on the conscious perception of target.
Thus far, we have analyzed data from five Os. We investigated the effects of external (stimulus properties), and internal (correctness, confidence) factors. In VD, HR decreased in anticipation of a target but the change from baseline decreased if detection was correct (hits, correct rejects). HRV increased due to an OR, indicating para activation, and again, change from baseline was smallest on hits. PEP increased as well, indicating sym deactivation, but no modulation by awareness was seen In VL, confidence modulated the ANS: HR changes from baseline were delayed and HRV changes from baseline were delayed and larger when the O was not confident. None of the factors modulated PEP. In summary thus far, the para, but not the sym, system, appears to be particularly sensitive to internal factors, i.e. confidence and correctness of visual perception. Being confident and correct in one's perceptual judgment reduces para fluctuations from baseline. Conscious perception dynamically modulates the body's systems, not just the brain. Consciousness carries consequences to body and brain.

Disclosures: B.R. Sheth: None. **Z. Li:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.12/PP23

Topic: D.04. Vision

Title: Pupil dilation reflects specific perceptual alternations in a bistable visual illusion

Authors: *N. A. KLOOSTERMAN[1,2], T. MEINDERTSMA[2], A. M. VAN LOON[1,2], T. H. DONNER[1,2];
[1]Dept. of Psychology, Univ. of Amsterdam, Amsterdam, Netherlands; [2]Cognitive Sci. Ctr. Amsterdam, Amsterdam, Netherlands

Abstract: Purpose: Pupil dynamics at constant illumination have been proposed as an index of phasic neuromodulation during perceptual decision-making. Specifically, pupil dilation has been observed during behavioral reports of perceptual transitions in bistable perceptual phenomena. It is unknown to which extent these pupil modulations reflect the contents of perceptual transitions and/or the motor act used to report these transitions. Asymmetric bistable visual illusions like **"**motion-induced blindness" (MIB) provide an opportunity to address these questions. In MIB, a salient and physically constant visual target surrounded by a rotating mask spontaneously disappears from visual awareness, only to reappear after a few seconds. Here, we linked pupil dynamics to the contents of these MIB transitions under various manipulations of behavioral report.
Methods: Pupil diameter was measured at constant luminance in 19 subjects. The static MIB target was a full-contrast Gabor patch (diameter: 2°) located in one of the four visual field quadrants (eccentricity: 5°). It was surrounded by a rotating mask (17°x17° grid of black crosses, speed: 120° or 160°/s), and superimposed on a gray background. In different conditions, subjects viewed either the continuous MIB stimulus or the intermittent physical removal of the target ("Replay"). Further, in different conditions, subjects were asked to report target disappearance by: 1) button press, 2) button release (each complementary for reappearance); 3) switching from one to the other button press; or 4) counting and reporting the total number after each run (Replay only). The overall pupil modulation around report was quantified by linear projection of single-trial time courses onto the mean time course.
Results: The pupil constricted during an interval of ~1 s before report and dilated during ~ 1 s after report. The overall pupil modulation amplitude was larger around disappearance than reappearance reports. This difference was driven by the constriction phase, and it was larger during MIB than Replay, and independent of motor regimen. This difference was also evident in the counting condition, in the absence of any overt behavioral report.
Conclusions: The neuromodulatory systems controlling pupil diameter have access to detailed information about bistable perceptual transition processes: Pupil modulation (in particular the constriction phase) reflects the contents of perceptual transitions, irrespective of the occurrence and type of motor action used for behavioral report. The pre-report constriction and post-report dilation may be driven by central cholinergic and noradrenergic mechanisms, respectively.

Disclosures: N.A. Kloosterman: None. **T. Meindertsma:** None. **A.M. van Loon:** None. **T.H. Donner:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.13/PP24

Topic: D.04. Vision

Title: Pupil dilation reflects the dynamics and content of a perceptual decision

Authors: *T. H. DONNER, J. W. DE GEE, T. KNAPEN;
Psychology, Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Background: Pupil dilation at constant illumination has been proposed as an index of phasic neuromodulation during decision-making. However, the exact nature of the link between pupil dynamics and decision-making is unknown. Simple perceptual decision tasks require the accumulation of noisy "sensory evidence" towards a categorical choice. They provide a well-controlled approach for linking pupil dynamics to the specific elements of a decision.
Purpose: We aimed to determine whether pupil dilation during perceptual decision-making reflects (i) the accumulation of sensory evidence during decision formation, or the final commitment to a choice; (ii) the content or the accuracy of the choice; and (iii) the decision-maker's intrinsic bias.
Methods: Pupil diameter was measured in 27 subjects performing a yes-no visual contrast detection task at constant luminance. On half the trials, only dynamic noise was presented (contrast: 5%). On the other half of trials, a low-contrast target pattern (contrast adjusted to the individual 75% correct threshold; range: 0.41% - 0.50%) was added to the noise. On each trial, subjects had to accumulate the "noisy evidence" and then choose between two button presses to report "yes, target seen" or "no, target not seen" (free response paradigm). Trials were sorted into hits and misses (target present trials), and false alarms and correct rejects (target absent trials). We used a general linear model (based on a canonical pupil impulse response function) to decompose decision-related pupil time courses into four components: transient on decision onset, sustained up- or down-ramp during decision formation, and transient on final choice. We quantified the overall decision-related pupil modulation by linear projection of single-trial pupil time courses onto the mean pupil response across all trials, aligned to the time of choice.
Results: All subjects exhibited prolonged decision times (range of median RT: 1002 - 2440 ms). The pupil exhibited significant positive transient components at decision onset and final choice, and a sustained up- (but not down-) ramp during decision formation. The overall modulation amplitude reflected the content, but not the accuracy, of the final choice: hits = false alarms > misses = correct rejects. The effect was stronger in the more conservative decision-makers (median split of subjects based on decision criterion).
Conclusions: We infer that the neuromodulatory systems controlling pupil diameter have access to rich information about ongoing decision processes: Pupil dilation reflects the dynamics and contents of decisions, and it is boosted when the decision-maker overrules her intrinsic bias.

Disclosures: T.H. Donner: None. **J.W. de Gee:** None. **T. Knapen:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.14/QQ1

Topic: D.04. Vision

Support: AFOSR/AFRL Grant FA9550-10-1-0385

Title: Certainly you're wrong: A dissociation between neural correlates of stimulus discrimination and confidence in perceptual decisions

Authors: G. BUZZELL[1], J. R. FEDOTA[1], D. M. ROBERTS[1], E. SHAW[1], *C. G. MCDONALD[2];
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Abstract: The occipital-temporal N1 component of the event-related potential (ERP) has been shown to index a visual discrimination process. Given that this component is enhanced when stimulus discrimination is difficult (despite controlling for low-level stimulus features), its modulation is thought to reflect a top-down control process. Moreover, error trials are associated with a reduced N1 component, suggesting that lapses in top-down control lead to a failure of this early discrimination process and incorrect stimulus categorization. However, this assumption has not been explicitly tested. In addition, while there is support for the notion that the P3b indexes the sensory evidence available for perceptual decisions, the relationship between early sensory processes and evidence-based categorization processes remains unclear. The present study employed a difficult perceptual discrimination task designed to clarify the relationship between these processes. In addition, participants were required to provide a subjective rating of stimulus certainty after each trial, allowing for the evaluation of perceptual decision confidence. As expected, the N1 component was diminished on error trials as compared to correct trials, even when only error trials in which participants were uncertain of stimulus identity were analyzed. However, the N1 did not differ as a function of stimulus certainty on correct trials. In contrast, an exploratory analysis revealed that an early (210-230 ms) frontal positivity was sensitive to stimulus certainty, this component being diminished when stimulus identity was uncertain on both correct and error trials. Therefore, the present results suggest the occipital-temporal N1 indexes the accuracy of stimulus discrimination (but not certainty), whereas an early frontal positivity indexes stimulus certainty (but not accuracy). The amplitude of the P3b component predicted both accuracy and stimulus certainty. Collectively, the present findings suggest that

there are separable processes underlying stimulus discrimination and certainty of stimulus identity. We suggest that the interaction of these processes determines the outcome of perceptual decisions.

Disclosures: G. Buzzell: None. **J.R. Fedota:** None. **C.G. McDonald:** None. **D.M. Roberts:** None. **E. Shaw:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.15/QQ2

Topic: D.04. Vision

Support: Royal Society

Usher Cunningham Studentship (DPAG and Exeter College, Oxford)

Title: Brain activity preceding internally generated switches in perception of a bistable structure-from-motion visual stimulus: A MEG study

Authors: *K. KRUG[1], S. BRAEUTIGAM[2], J. HEATH[1], A. J. PARKER[1], N. CICMIL[1];
[1]Dept Physiol, Anat & Gen, Oxford Univ., Oxford OX1 3PT, United Kingdom; [2]Dep Psychiatry, Oxford Univ., Oxford OX3 7JX, United Kingdom

Abstract: Perceptually bistable stimuli probe the neural mechanisms by which the brain resolves ambiguous sensory input (Kleinschmidt et al, 2012). During prolonged viewing, observers' perception 'switches' between possible interpretations although the stimulus does not change. Recent studies with apparent motion and Ruben's face-vase stimuli implicate both frontal and sensory networks in this process (Sterzer et al, 2009). However, the timing of different neuronal activity patterns preceding such perceptual switches has not been fully elucidated.
We measured brain activity with magnetoencephalography (MEG) in 9 volunteers as they judged the rotation direction of an ambiguous structure-from-motion cylinder. Recordings were taken in 5 blocks, each of 10 mins duration. In blocks A-D, a perceptual switch in rotation direction was externally induced by applying binocular disparity to separate the cylinder's front and back surfaces, at 12 to 82 second intervals for durations of 4 to 12 seconds. Disparities were matched to individuals' psychophysical thresholds for 95% correct responses. In block E, no disparity was applied so rotation direction was bistable. Block order was randomized across participants, who gave a left or right button press for clockwise or counter-clockwise rotation respectively.
MEG signals from 204 gradiometers were spatially aligned across participants, filtered at 1-30

Hz and epoched at -2000 ms to 0 ms pre-button press. Artefactual epochs were rejected. Statistical activity differences across sensors between internal and external switch conditions (paired Wilcoxon test of matched samples) were compared at group level as a function of time, following a $\chi 2$ distribution (Braeutigam et al, 2004).
We found significant differences in brain activity that preceded internally versus externally generated switches at around -1675 ms, -1490 ms, -1250 ms, -1170 ms, -1130 ms and -1015 ms ($\chi 2$ test, n=9, $p<0.001$). When internal switches were compared to baseline, significant activity occurred at -1665 ms, -1490 ms, -1290 ms, -1090 ms and -985 ms ($p<0.01$). Group results were confirmed by individual-subject statistics (Mann-Whitney-U test, $p<0.01$). Topologically, differences in right hemisphere frontal and parietal regions were seen early in the internal switch condition (-1665 ms).
In summary, activity unique to internally generated SFM perceptual switches is evident more than 1500 ms prior to the associated behavioral change. Activity patterns at this time implicate a contribution of fronto-parietal regions to internally generated switches whilst early activity in posterior regions appears to be more specific to externally induced switches.

Disclosures: K. Krug: None. **S. Braeutigam:** None. **J. Heath:** None. **A.J. Parker:** None. **N. Cicmil:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.16/QQ3

Topic: D.04. Vision

Title: The role of synaptic depression in a biologically plausible model for percept choices at the onset of ambiguous visual stimuli

Authors: *R. VAN WEZEL[1], C. KLINK[2], W. WOLDMAN[3], M. TE WINKEL[1], S. VAN GILS[3], H. MEIJER[3];
[1]Biophysics, Radboud University, Donders Inst. For Brain, Cognition and Behaviour, Nijmegen, Netherlands; [2]Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [3]Mira Inst. for Biomed. Technol. and Tech. Med., Enschede, Netherlands

Abstract: Visual percept choices for sequences of repeated ambiguous stimuli depend on the time interval between subsequent stimulus presentations. Short blank intervals cause the percept to alternate, while at longer intervals the percept stabilizes into a single perceptual interpretation (perceptual memory). Here we show a biologically plausible computational model that describes

the dynamics of these choice dynamics. The model consists of excitatory and inhibitory tuned neurons and it includes, cross-inhibitory interactions, spike adaptation (with a short time constant) and synaptic depression (with a long time constant). Simulations of the model are consistent with our previous human psychophysical and neurophysiological experimental data. The model predicts that adaptation and synaptic depression deterministically determine the transition from alternating to repeated percepts in sequences of ambiguous stimuli. Our model shows that no explicit (higher-order) memory or facilitatory component is necessary to explain these memory effects in visual perception.

Disclosures: R. Van Wezel: None. **C. Klink:** None. **W. Woldman:** None. **H. Meijer:** None. **S. van Gils:** None. **M. te Winkel:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.17/QQ4

Topic: D.04. Vision

Support: The Kavli Institute for Brain and Mind (KIBM)

JFM

CTM

JHR

Title: Head-restrained marmosets discriminate fine differences in orientation

Authors: *C. P. CHOW[1], J. H. REYNOLDS[2], C. T. MILLER[1], J. F. MITCHELL[2]; [1]Psychology, UCSD, La Jolla, CA; [2]Systems Neurobio., The Salk Inst., La Jolla, CA

Abstract: The small New World monkey, the common marmoset (Figure 1E), offers several advantages for studies of visual neurophysiology. They are lissencephalic, making them ideally suited for laminar recording and the recent development of transgenic lines (Sasaki et al, 2009) raises the possibility of cell-type-specific expression of proteins, such as opsins and genetically encoded calcium indicators. While the anatomy and physiology of the marmoset visual system has been studied extensively in anaesthetized animals (Rosa et al, 2009), it remains unclear if they can perform visual tasks with the head fixed. This will be crucial both for accurate eye tracking and standard neurophysiological methods. We tested if a marmoset could discriminate the location of an oriented Gabor that differed in orientation from distracters. Each trial was

initiated when the marmoset fixated a small flashing central point (Figure 1A). After holding fixation at the center for a variable period (Figure 1B), six equally spaced gratings (2 degrees in diameter, Gabor σ =0.5 degrees, spatial frequency varying from 1, 1.4, or 2 cycles/deg, random spatial phase) were presented at 5 degrees eccentricity (Figure 1C). Five of the six gratings were horizontally oriented while one was tilted (+/- 0, 2, 4, 8, 12, 16, 32, or 45 degrees). After 250 ms, the fixation point disappeared cueing the marmoset to make a saccade to the location of the tilted grating (Figure 1D). The marmoset was rewarded for holding fixation and then rewarded again for correctly indicating the target location, with reward increasing with task difficulty. The spatial frequency, tilt, and target location were chosen at random each trial. Performance was above chance at 4 degrees and >80% correct at 16 degrees (Figure 1F). Reaction times increased with difficulty (Figure 1G). These results establish the marmoset as a viable model for studies of visual perception.

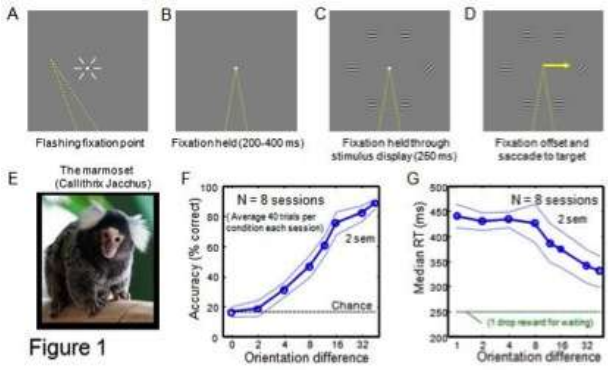

Figure 1

Disclosures: C.P. Chow: None. **J.H. Reynolds:** None. **C.T. Miller:** None. **J.F. Mitchell:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 262.18/QQ5

Topic: D.04. Vision

Support: The Kavli Institute for Brain and Mind (KIBM)

Title: Developing awake behaving marmosets as a model for visual neuroscience

Authors: *J. F. MITCHELL[1], J. H. REYNOLDS[1], C. T. MILLER[2];
[1]Salk Inst., LA JOLLA, CA; [2]Psychology, UCSD, La Jolla, CA

Abstract: Our understanding of the neural mechanisms underlying perception and cognition has been limited by the lack of tools to modulate the activity of specific neural circuits in the awake

animal. Recent advances in optogenetics have begun to make this level of control possible in the mouse. However, mice are difficult to train, and their brain differs substantially from the primate brain. While the rhesus macaque has been the model system of choice for studying perception and cognition, smaller New World primates, such as the marmoset (Callithrix jacchus), offer many advantages including the potential to develop transgenic lines (Sasaki et al, 2009). The anatomical layout of visual areas and the properties of their visual neurons, recorded under anesthesia, are highly homologous to that of the macaque (Rosa et al, 2009). Detailed maps are available for their visual and oculomotor control areas, providing a sound basis for continued study in the awake animal. However, a critical unknown is whether marmosets can perform behavioral tasks when the head is stabilized, which facilitates neuronal recording and is critical for accurate measurement and control of eye movements. We replicated techniques for training macaques in fixation and visual tasks, but on a smaller scale appropriate for their smaller size. Here we show that in free viewing marmosets actively explore natural scenes with saccadic eye movements and target regions of interest such as faces (Figure 1A and B). More importantly, we show that they can control their fixation for liquid reward (see demo at http://www.snl.salk.edu/~jude/marmofix.MOV). We are currently investigating the receptive field properties of their visual cortical neurons using 16 channel laminar probes. Laminar recordings are particularly well suited for the marmoset due to their lissencephalic (flat) cortex. This enables perpendicular placement of the probes through the cortex even in extra-striate areas that would fall in sulci of macaques, which facilitates the identification of layer in recordings using current source density (CSD) analysis.

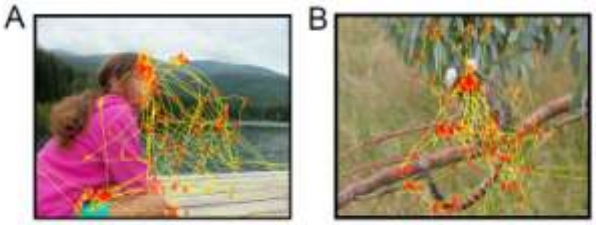

Figure 1

Disclosures: J.F. Mitchell: None. **J.H. Reynolds:** None. **C.T. Miller:** None.

Poster

262. **Visual Decision Making**

Location: Halls B-H

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Topic: D.04. Vision

Support: Grants- in-Aid for Scientific Research 24300146, Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT)

Title: Humans exploit uncertainty and bimodality of priors in motion direction estimation

Authors: *S. LAQUITAINE, J. L. GARDNER;
Gardner Res. Unit, Riken Brain Sci. Inst., Wakoshi, Japan

Abstract: What aspects of prior information do humans exploit when estimating perceptual quantities like motion direction? Bayesian inference suggests biasing towards the mean of the prior set of directions when sensory evidence is made weak by decreasing the coherence of motion. But certainty of the prior (i.e. width) should also affect how much to weight the prior mean in perceptual estimates. Moreover, there may be more than one single likely direction and so shape of the prior distribution (unimodal or bimodal) should also affect estimates. We used a motion direction estimation task and found that humans were sensitive to both changes in the width and shape of the prior.

Sensitivity to prior width was tested by changing the distribution of directions that subjects were asked to estimate. Four subjects performed a task in which a patch of random dots (5 deg diameter, 16.7 dots/deg, circular aperture) moving at 2.8 deg/sec in 36 different directions (5-355 deg evenly spaced) with 3 motion coherences (6, 12 and 24%) were shown for 0.3 secs in randomized order. Subjects reported the direction of motion by adjusting the orientation of an arrow with an electronic paddle wheel. At trial end, the true motion directions were displayed to subjects though they were not explicitly given feedback about correctness. Displayed directions were drawn randomly from 1 of 4 discrete Gaussian prior distributions with different standard deviations (10, 20, 40 and 80 degs) but the same mean. We found that subjects were biased toward the most likely direction and that this effect was strongest when the prior width was smallest (i.e. most certain) ($F(3, 177) = 60.528$, $p<0.001$, ANCOVA). These data were well fit by a Bayesian model in which likelihood and prior distributions were gaussian with means set by experimental parameters and standard deviations were model parameters ($R2 = 0.98$), showing that subjects tracked the width of the prior.

Sensitivity to prior shape was tested by creating a bimodal distribution of prior directions. The experimental design was the same except displayed directions were sampled from one of four discrete bimodal prior distributions (means of the two modes: 145 and 305, 165 and 285, 185 and 265, 205 and 245 degs) all with the same overall mean. We found that although each of the priors had the same overall mean, subjects' estimates changed depending on which set of bimodal distributions were used showing that performance tracked changes in the shape of the prior.

Altogether our results show that humans exploit complex aspects of prior knowledge such as the width (uncertainty) and shape (bimodality) of priors to improve direction perception, consistent with Bayesian inference.

Disclosures: S. Laquitaine: None. **J.L. Gardner:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.01/QQ7

Topic: D.05. Visual Sensory-motor Processing

Support: R01-MH099611

R01-EY017366

Title: Precise characterization of dorsal stream neural activity during decision making

Authors: *J. YATES, I. PARK, L. K. CORMACK, J. PILLOW, A. C. HUK;
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Abstract: The accumulation of noisy sensory evidence is a necessary step in decision making. Over three decades of research recording from neurons in the primate brain during a motion discrimination task have suggested a circuit model where neurons in parietal area LIP integrate motion information encoded by neurons in MT. We sought to test this model directly by recording from populations of cells in MT and LIP simultaneously while a monkey performed a novel reverse-correlation motion discrimination task where the direction of local motion energy is precisely and independently varied over time. The reverse correlation stimulus consists of a series of motion pulses presented for 1,116ms on each trial. The pulses consist of a field of small flickering gabor patches. Each pulse lasts 166ms, during which a number and direction are drawn at random and that number of gabors drift in the chosen direction. This variable presentation allows us to measure how temporal variations in motion strength correlate with both the monkey's perceptual decision and the spiking activity of single neurons. We recorded 17 sessions from LIP (44 cells, 2-8 cells per session). Using Bayesian logistic regression to classify the monkeys choices and a generalized linear model (based on Poisson regression) to fit single trial spike trains, we were able to recover and compare the temporal weighting function of both monkey and neuron(s) within individual experimental sessions. We found the temporal weighting of the motion stimulus in LIP is consistent with the monkey's psychophysical weighting, although the effect of a single motion pulse on the spiking activity in LIP was considerably more complex, implying an indirect relation between LIP and decisions. Furthermore, we often observed fine timescale cross-correlations between neurons with overlapping RFs that were time- and decision-dependent, suggesting shared input. Analysis of the pulse-triggered average for MT neurons confirms that they represent the motion stimulus with high fidelity, in a manner that is less related to the eventual decision than in LIP. These

results reveal the feasibility of testing hypothesis of sensorimotor transformations during motion decisions at the level of single trials, using neural activity that spans multiple brain areas.

Disclosures: J. Yates: None. **I. Park:** None. **L.K. Cormack:** None. **J. Pillow:** None. **A.C. Huk:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.02/QQ8

Topic: D.05. Visual Sensory-motor Processing

Support: NIH Grant EY10562

Marsden Fund

Title: Tuning of MST neurons during active steering

Authors: *S. W. EGGER[1], K. H. BRITTEN[2];
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Abstract: Neurons in the medial superior temporal sulcus (area MST) are hypothesized to guide navigation to a goal. In passive sensory tasks neurons in MST prefer complex motion stimuli similar to that observed during self-movement, suggesting MST neurons process optic flow for the purpose of recovering the components of self-motion. However, no one has measured the responses of MST neurons during active navigation tasks, leaving open the question of MST's role in guiding locomotion. To test if their responses can support active navigation, we recorded from single MST neurons as a monkey used a joystick to steer to a distant target in a virtual world. In this task, performance depends on two cues: the target direction and the optic flow. Because the stimuli viewed during each trial was different, traditional tuning measurements could not be employed to assess MST responses. We therefore developed a novel method for determining the tuning function of the neurons from individual trials. Using a smoothing function to estimate the underlying activation function of individual MST neurons, we found the tuning of each neuron based on the activation function, conditioned on the past values of different steering stimuli. From this we measured the tuning surface over the optic flow and target location cues. As expected, many MST neurons exhibited tuning to the large motion field experienced during simulated rotations of the monkey. More surprisingly, MST neurons also exhibited tuning to the location of the small target onscreen. Recording the stimulus as viewed during active steering, we were able to replay the same steering stimulus to the monkey multiple

times. The responses of MST neurons fired in a similar pattern, both to repeats of the replay stimulus and during active steering. These results allowed us to validate our methodology for determining the underlying tuning from individual trials. Taken together, our observations support the role of MST in guiding navigation, but suggest the role of MST is more complex than previously thought.

Disclosures: S.W. Egger: None. **K.H. Britten:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.03/QQ9

Topic: D.05. Visual Sensory-motor Processing

Support: F32-EY019851

R01-EY08890

P30-EY08126

P30-HD015052

E. Bronson Ingram Chair in Neuroscience

Title: Network configuration for task preparation in prefrontal cortex

Authors: *R. P. HEITZ, J. D. SCHALL;
Vanderbilt Univ., NASHVILLE, TN

Abstract: Nonspecific preparation can occur when an underdetermined action will be produced following some cuing event, such as a warning signal. Typically, nonspecific preparation is studied through the use of a variable foreperiod, beginning with some warning signal and ending with an imperative stimulus that requires perceptual analysis and overt response. A history of research indicates that such warning signals increase performance, so long as foreperiods are not excessively long or short. Despite decades of research, the neural mechanisms subserving nonspecific preparation have been limited to components of the EEG (e.g., the readiness potential over motor cortex).
Here, we explored the single-unit neural correlates of nonspecific preparation in macaque monkeys performing a difficult T/L visual search experiment. Trials began with the appearance of a small fixation point which monkeys were to fixate and maintain gaze. This foreperiod

ranged from 750 to 3000 ms drawn from a uniform distribution. No stimuli other than the fixation point were present during the foreperiod. Following the foreperiod, a visual search array appeared containing 1 target and 7 distractors; monkeys made saccades to the target to earn juice reward. Additionally, we included a speed-accuracy tradeoff manipulation, signaled by the color of the fixation point, which indicated whether the impending stimulus required a very fast or very accurate response.

We recorded the activity of single neurons in the frontal eye field (FEF), a region of prefrontal cortex integral to perceptual analysis, decision formation, and eye movement execution. We found that over the course of the foreperiod, absolute spiking rates were invariant, suggesting that the overall neural output did not change. However, measures of across-trial neural variability (Fano factor) declined progressively. Moreover, the decline was exaggerated when monkeys were instructed to respond more quickly to the upcoming stimulus than when instructed to respond more accurately.

These results suggest that nonspecific preparation is accompanied by a decrease in neural variability. This is consistent with an evolution of network activity into an optimal subspace to increase readiness. Such a network configuration may be driven by neural discharge modulation in medial frontal areas and could stem from increasing entrainment of neural networks through coherence.

Disclosures: R.P. Heitz: None. **J.D. Schall:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.04/QQ10

Topic: D.05. Visual Sensory-motor Processing

Title: Bilateral modulation of motor systems by the stimulated visual field in an inter-hemispheric transfer task

Authors: *A. A. OMARI[1], V. DIWADKAR[1], R. WHITE[1,2], G. RAMBALDELLI[2], M. BELLANI[2], S. SAVAZZI[2], C. MARZI[2], P. BRAMBILLA[2];
[1]Dept. of Psychiatry and Behavioral Neurosciences, Wayne State Univ. Sch. of Med., Detroit, MI; [2]Med. and Publ. Hlth., Univ. of Verona, Verona, Italy

Abstract: Introduction: Inter-hemispheric transfer has been assessed using the Poffenberger paradigm (Marzi, 1999), a task that depends on uni-manual responses to probes presented to either visual cortex. In general, regardless of response hand, the “crossed” condition, that is the

condition when visual information is presented to a different hemisphere than the response hand, results in increased response latency and increased cortical and callosal activations (Tettamanti et al., 2002). To examine whether the stimulated visual cortex modulated ipsi- and contra-lateral motor cortex during the crossed condition, we used psycho-physiological interaction (PPI; Friston et al., 1997).

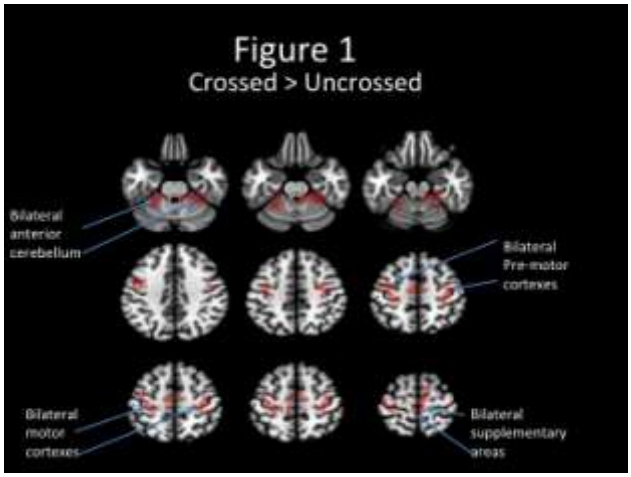
Methods: fMRI (3T, Siemens Magentom Allegra) was acquired in sixteen healthy right-handed subjects between the ages of twenty and fifty. During the task, a probe was briefly presented in one of two visual hemi-fields and subjects responded with the left or right hand. Only right-handed responses were analyzed. PPI maps were constructed convolving time series from the excited visual cortex (right) within the contrast of interest (Crossed > Uncrossed). The maps, encoding modulation by the visual cortex, were submitted to a second level analysis to identify clusters of significant modulation.

Results:

Figure 1 depicts a mosaic of axial views with clusters showing significant ($p<.05$) modulation by the stimulated visual cortex. Clusters were observed bilaterally in the anterior cerebellum, pre- and primary motor cortex, supplementary motor cortex and bilateral primary motor cortexes.

Discussion:

These results are the first to suggest engagement of bilateral brain networks by the stimulated visual hemisphere during an inter-hemispheric visuo-motor transfer paradigm. Cross-hemispheric information transfer during the Poffenberger task may result from parallel signaling by the stimulated visual hemisphere of bilateral cortical and sub-cortical motor networks.



Disclosures: A.A. Omari: None. **V. Diwadkar:** None. **R. White:** None. **G. Rambaldelli:** None. **M. Bellani:** None. **S. Savazzi:** None. **C. Marzi:** None. **P. Brambilla:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.05/QQ11

Topic: D.05. Visual Sensory-motor Processing

Support: FRS-FNRS

Title: Influence of top-down control on selective attention and response selection during motor decision making

Authors: *P.-A. KLEIN, A. ZÉNON, A. MOURAUX, S. NOZARADAN, J. DUQUE;
Univ. Catholique De Louvain, Brussels, Belgium

Abstract: At every moment, the environment presents us with multiple stimuli that call for attention and action. We continuously have to attend to and act towards stimuli which are the most compatible with our goals, while refraining from selecting less relevant, yet possibly appealing, options. Decision making involves at least two major interrelated processes. The first one, called “selective attention”, biases competitive interactions between sensory stimuli by favoring processing of information that is relevant to specific goals while ignoring irrelevant stimuli in the environment. The second process, “response selection”, is associated with the accumulation of activity in competing response representations so as to ensure that only motor activity related to the most beneficial action reaches threshold and is selected. Here, we used electroencephalography (EEG) to characterize the interactions between selective attention and response selection. Participants performed a version of the Flanker task in which they were asked to indicate by a left or right button-press the orientation of a briefly presented left- or right-facing central arrow, flanked by a set of two distractor arrows on each side which either pointed in the same (congruent trials) or in the opposite -conflicting- direction (incongruent trials). The proportion of congruent and incongruent trials was manipulated to produce two different contexts in terms of conflict expectation (mostly congruent [MC]: 80% congruent trials; mostly incongruent [MI]: 80% incongruent trials). Subjects expected more conflict in the MI compared to the MC. Selective attention and response selection processes were assessed in 12 subjects by assessing steady-state visual evoked potentials (SSVEP, central and distractor arrows flickered at different frequencies) and current source density (CSD) amplitudes over the relevant and irrelevant motor representations. Response times and error rates were larger in incongruent compared to congruent trials. However, this difference was attenuated in MI compared to MC, probably because control mechanisms were recruited in MI to help sharpen selective attention and response selection. SSVEPs indicate a larger processing of the central arrow in MI than MC, with no change observed for the distractor arrows. This increased selective attention was associated with larger CSD amplitude over the relevant motor representation in MI, mainly in incongruent trials. The role of specific parieto-frontal areas in selective attention and response

selection is currently under investigation by means of a combined EEG and repetitive transcranial magnetic stimulation protocol.

Disclosures: P. Klein: None. **J. Duque:** None. **A. Mouraux:** None. **S. Nozaradan:** None. **A. Zénon:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

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Topic: D.05. Visual Sensory-motor Processing

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SNF Grant 31003A-118069

Zurich Center for Integrative Human Physiology (ZIHP)

Betty and David Koetser Foundation for Brain Research

Title: Velocity storage mechanism in zebrafish larvae

Authors: *Y.-Y. M. HUANG[1], C.-C. CHEN[1], C. J. BOCKISC[1], I. OLASAGASTI[1], G. BERTOLINI[1], S. NEUHAUSS[2], K. P. WEBER[1], D. STRAUMANN[1];
[1]Neurol. Department, Zurich Univ. Hosp., Zürich, Switzerland; [2]Imls, Univ. of Zurich, Zurich, Switzerland

Abstract: The optokinetic reflex (OKR) and the angular vestibulo-ocular reflex (aVOR) complement each other to keep the line of sight stable despite of self- or world-motion , a joint mechanism that is critical for effective vision. It is currently hypothesized that signals from both systems integrate in a network of neurons operating as a velocity storage mechanism (VSM). When exposed to a rotating visual surround, subjects display the OKR, slow following eye movements frequently interrupted by fast resetting eye movements. Subsequent to light-off during optokinetic stimulation, eye movements do not stop abruptly, but decay slowly, a phenomenon referred to as the optokinetic after response (OKAR). The OKAR is most likely generated by the VSM. In this study, we observed the OKAR in larval zebrafish before the developmental emergence of the angular VOR. Our results suggest that the neural structures underlying the VSM may be already functional prior to being primarily regulated by angular vestibular signals. The VSM may be critical to ocular motor control in early development as it increases the efficiency of the OKR.

Disclosures: Y.M. Huang: None. **C. Chen:** None. **C.J. Bockisc:** None. **I. Olasagasti:** None. **G. Bertolini:** None. **S. Neuhauss:** None. **K.P. Weber:** None. **D. Straumann:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

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Topic: D.05. Visual Sensory-motor Processing

Support: NIH Grant P01HD064653

Title: The visual discharge of mirror neurons in monkey PMv is modulated by the gaze direction of the observed agent

Authors: *G. COUDE[1], F. FESTANTE[1], A. CILIA[1], V. LOIACOMO[1], M. BIMBI[1], L. FOGASSI[2], P. FERRARI[3];
[1]Univ. Parma, Dept Neurosci., Parma, Italy; [2]Univ. Parma, Dept Psychology, Parma, Italy; [3]Univ. of Parma, Dept Biol., Parma, Italy

Abstract: Mirror neurons (MNs) in the inferior parietal lobule (IPL) and ventral premotor cortex (PMv) can code the intentions of other individuals using contextual cues. Gaze direction is an important social cue that can be used for understanding the meaning of actions made by other individuals. It is known that nonhuman primates are sensitive to others' head and gaze orientation. Here we addressed the issue of whether PMv MNs are influenced by the gaze direction of another individual. We recorded single-unit activity in macaque PMv while the monkey was observing a human subject performing a grasping action while orienting his/her gaze toward (congruent condition) or away (averted condition) from the target object. Grasping could be performed either in the left or the right hemispace relative to the recorded hemisphere. We recorded 82 PMv MNs and found that 30 (36%) of them were modulated by the gaze direction of the human agent. Out of them, 12 (40%) of the neurons had a stronger firing rate during the congruent condition than during the averted one, while 18 (60%) of them preferred the averted condition. These preliminary results indicate that the discharge of a population of MNs in PMv is modulated by gaze direction. Since MNs modulated by gaze were also found in IPL, it is likely that MNs in PMv receive information concerning gaze direction from parietal cortex and, together with this latter cortical sector, use this information for decoding the intention of other individuals. The representation of actions in PMv could therefore be influenced by contextual information not only extracted from physical cues, but also from cues of a biological or social nature.

Disclosures: G. Coude: None. **F. Festante:** None. **A. Cilia:** None. **V. Loiacomo:** None. **M. Bimbi:** None. **L. Fogassi:** None. **P. Ferrari:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.08/QQ14

Topic: D.05. Visual Sensory-motor Processing

Support: University of Crete, Special Account for Research, Grants 3704 and 3767

Title: Cue-dependent action-observation elicited responses in the ventral premotor cortex (area F5) of the macaque monkey

Authors: *V. PAPADOURAKIS[1,2], V. RAOS[1,2];
[1]Dept of Basic Sci., Univ. of Crete Med. Sch., Iraklion, Greece; [2]FORTH/IACM, Iraklion, Greece

Abstract: Our aim was to re-investigate the visual and motor properties of F5 mirror neurons (MNs), using a controlled paradigm in order to correlate the neuronal discharge with temporal events of the observed movement.
Initially, the monkey was trained to reach for and grasp 3D objects with the appropriate grips. At the beginning of each trial, a LED above the selected object turned on and the monkey had to fixate it and press a key. Following a fixation period, a dimming of the LED signaled the onset of the reach-to-grasp movement. The monkey had to reach for, grasp, pull and hold the object while fixating it until the extinction of the LED cuing its release. Then, the monkey was trained to observe the experimenter employing two variations of the above task (randomly interleaved) while maintaining its gaze straight ahead (OBS). In the first, the cuing LED was visible to the monkey (CUEOBS) whereas, in the second, the LED was off and the experimenter was getting instructions on a screen out of the monkey's view (NOCUEOBS). Moreover, a LED fixation condition in which the experimenter was not performing any movement was used as a control. Both forelimbs of the monkeys were gently restricted during the OBS conditions. The experimenter was standing next to the animal on its right side, and both reaching and grasping components of his movement were visible to the monkey.
We recorded 147 neurons responding to action observation. Out of them, 110 were tested also at the execution task and displayed motor discharge. Hierarchical cluster analysis of the observation-driven net (-spontaneous) normalized response profile (% of the max) in the CUEOBS, NOCUEOBS and LED conditions revealed two main, almost equally populated,

classes of neurons. The response of the neurons belonging to the 1st class starts after the onset of the movement, reaches its maximum when the experimenter's hand contacts the object and returns to baseline either during or at the end of the object-holding period, displaying an almost identical profile in the two OBS conditions. These neurons are similar to the MNs described by Rizzolatti and coworkers. In contrast, the response profile of the neurons in the 2nd class exhibits temporal differences between the two OBS conditions. In the NOCUEOBS the discharge starts with the beginning of the movement whereas in the CUEOBS the firing starts several hundreds of ms before it. The response reaches its maximum at the midway of the movement (with the peak in CUEOBS preceding that in NOCUEOBS in many cases) and returns to baseline soon after the object is grasped.
These results provide new elements about the response-triggering features of MNs and pave the way to new considerations about their functions.

Disclosures: V. Papadourakis: None. **V. Raos:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.09/QQ15

Topic: D.05. Visual Sensory-motor Processing

Title: Effect of aging on inhibitory mechanisms involved in movement preparation

Authors: *C. PETITJEAN;
Univ. Catholique Louvain (UCL), Brussels, Belgium

Abstract: Top-down control is critical to select goal-directed actions, particularly when several conflicting options compete for selection. This control is thought to involve a mechanism that suppresses activation of unwanted response representations. The aging population shows a reduced ability to make appropriate decisions, especially when they are faced with conflicting information. Here, we tested the hypothesis that this alteration is due to a deterioration of inhibitory control regarding unwanted response representations.
Twelve young and ten elderly participants performed an Eriksen Flanker task in which they were asked to indicate by a left or right button-press the orientation of a briefly presented central arrow, flanked by a set of two distractor arrows on each side. The central and distractor arrows either pointed in the same (congruent trial) or in the opposite - conflicting - direction (incongruent trial). The proportion of congruent and incongruent trials was manipulated to produce two different contexts in terms of conflict expectation (mostly congruent [MC] context:

80% congruent trials; and mostly incongruent [MI] context: 80% incongruent trials). Obviously, subjects expected more conflict in the MI compared to the MC context. Motor evoked potentials (MEPs) were recorded from the left first dorsal interosseous muscle (FDI), an index finger agonist in the task, following single pulse transcranial magnetic stimulation (TMS) over the right primary motor cortex (M1). MEPs were elicited at one of two possible timings during movement preparation and compared to MEPs elicited at baseline (fixation cross presentation). This procedure provided us with a measure of corticospinal excitability change associated with a selected (during preparation of a left response) or non-selected (right response) muscle in congruent or incongruent trials, in a MI or MC context.
The behavioural data indicate that elderly subjects were slower but made less errors than young subjects. Aside from this, all subjects were slower and performed more errors in incongruent compared to congruent trials. However, both groups displayed a better performance in incongruent trials of the MI than MC context suggesting the recruitment of additional top-down control resources when conflict was expected in advance. In young subjects, MEPs were particularly inhibited in the MI context, suggesting that suppression of motor activity is an important component of conflict resolution. In contrast, in elderly subjects, MEPs were found larger in the MI context. These results suggest a different pattern of MEP changes during movement preparation under conflict in young and elderly subjects.

Disclosures: C. Petitjean: None.

Poster

263. **Sensorimotor Transformations: Physiology**

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Program#/Poster#: 263.10/QQ16

Topic: D.05. Visual Sensory-motor Processing

Support: NIH Pioneer 1DP1OD006409

REPAIR-N66001-10-C-2010

Burroughs Wellcome Fund Career Award

Howard Hughes Medical Institute

Fundacao para a Ciencia e Tecnologia

Champalimaud Neuroscience Programme

Title: Neural correlates of action choice and RT in dorsal premotor cortex

Authors: *C. CHANDRASEKARAN[1], D. PEIXOTO[2,3,6], W. T. NEWSOME[2,7], K. V. SHENOY[1,2,4,5];
[1]Electrical Engin., [2]Neurobio., [3]Sch. of Med., [4]Program In Neurosci., [5]Bioengineering, Stanford Univ., Stanford, CA; [6]Champalimaud Neurosci. Programme, Lisbon, Portugal; [7]Stanford Univ. / HHMI, Stanford, CA

Abstract: How does the primate brain make decisions based on sensory input and decide which action to perform when? Lesion studies suggest that dorsal premotor cortex (PMd) is involved in mapping sensory cues to actions. One action selection framework proposed for ambiguous circumstances is that PMd simultaneously encodes potential reach directions (Cisek & Kalaska, 2005). As information allowing for reach selection appears, populations of neurons selective for a reach direction compete and eventually one population wins, enhancing its firing rate over the other population and resulting in a reach. This makes three predictions. First, in ambiguous reach contexts, neurons in PMd selective for different reaches should initially respond regardless of eventual choice. Second, over time one selective population should enhance its firing rate, winning the competition and resulting in a reach. Third, the arm movement reaction time (RT) should closely follow the time course of neural competition. We tested these predictions in PMd of a monkey performing a visual choice RT task.
A trained monkey used his right arm to report the dominant color in a central static checkerboard composed of isoluminant red and green squares. The percentage of red and green in the stimulus varied from trial to trial. The monkey's behavior followed behavior typical of visual RT tasks; increases in difficulty led to more discrimination errors and slower RTs. Most RTs varied between ~400 ms for the easiest discrimination to ~550 ms for the hardest discriminations (range: 300 to 1000 ms). While the monkey performed this task, we recorded the activity of single neurons (70) and multi-units (19) from the arm region of left PMd.
We observed a mixed population of neural responses in PMd. Broadly, one subset of the population was movement sensitive (53/89), changing its firing rates just before (~200 ms) the reach onset. However, other neurons (36/89), resembling "visuomovement" cells responded rapidly after checkerboard onset regardless of the eventual choice. All three predictions were supported by data from visuomovement cells. Immediately after stimulus onset, cells selective for rightward and leftward reaches exhibited comparable firing rates. Over time, however, the absolute difference in activity of these two populations increased; achieving on average a 25 spikes/s difference (~100 ms) prior to movement onset. Importantly, the evolution of this activity difference was faster for fast RTs and slower for slower RTs. These results extend the competition hypothesis and suggest that dynamics in PMd neurons may mediate the selection of the action to perform and when to perform it.

Disclosures: C. Chandrasekaran: None. **D. Peixoto:** None. **W.T. Newsome:** None. **K.V. Shenoy:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.11/QQ17

Topic: D.05. Visual Sensory-motor Processing

Support: NIH AG031769

Title: Altered oscillations in EMG variability explain impaired ankle movement control in children during a high-gain visual feedback condition

Authors: *H. MOON, C. KIM, M. KWON, Y. CHEN, E. J. FOX, E. A. CHRISTOU;
Univ. of Florida, Gainesville, FL

Abstract:

It is well-established that high-gain visual feedback exacerbates motor control in older adults compared with young adults due to altered activation of the agonist muscle. Nonetheless, it is unknown whether high-gain visual feedback has similar effects on the motor control of children. The purpose of this study was to compare the effect of high-gain visual feedback on movement variability and muscle activation in children and young adults. Six young adults (19.8 ± 0.6 yrs) and 9 children (9.4 ± 1.6 yrs) participated in one session. Subjects attempted to accurately trace a sinusoidal target by performing ankle plantar/dorsiflexion movements. The targeted range of motion was 10^{o} and the frequency of the sinusoidal target was 0.4 Hz for 35 s. We examined movement control at a low-gain (0.66^{o}) and a high-gain (4.68^{o}) visual feedback condition. Surface EMG was recorded from the tibialis anterior muscle. Movement variability was quantified as the standard deviation of the position fluctuations after the task frequency was removed with a band-stop filter (2^{nd} order; 0.3-0.5 Hz). EMG variability was quantified as the EMG fluctuations of the rectified low-pass (2 Hz) filtered EMG after the task frequency was removed as described for movement variability. In addition, we quantified the power spectrum of the movement variability and EMG variability using the following frequency bands: 0-0.3, 0.3-0.6, 0.6-0.9, 0.9-1.2, and 1.2-1.5 Hz. Children exhibited greater movement variability compared with young adults for both visual feedback gains ($P<0.05$). This age-associated difference was exacerbated during the high-gain visual feedback condition ($P<0.05$). Children exhibited greater power in their movement variability from 0-0.6 Hz and lower power from 0.6-1.5 Hz compared with young adults ($P<0.05$). The amplified ankle movement variability in children at the high-gain visual feedback condition was predicted by lower power within the 0.6-1.5 Hz of their movement variability ($R^2=0.57$, $P<0.01$). Similarly, the amplified ankle movement variability in children at the high-gain visual feedback condition was predicted by greater power within 0.3-

0.6 Hz and lower power within 0.6-0.9 Hz of their EMG variability (R^2=0.48, P<0.01). In conclusion, children exhibited impaired ankle movement control compared with young adults, especially during the high-gain visual feedback condition. This impairment was largely explained by altered oscillations of movement variability and EMG variability of the tibialis anterior muscle. The observed age differences may be due to the immaturity of the cortico-motor systems.

Disclosures: H. Moon: None. **C. Kim:** None. **M. Kwon:** None. **Y. Chen:** None. **E.J. Fox:** None. **E.A. Christou:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.12/QQ18

Topic: D.05. Visual Sensory-motor Processing

Support: NIH R01 NS065065

Title: Neurons in monkey dorsal premotor cortex are weakly sensitive to eye position when gaze fixation is untrained

Authors: *B. ALEMAYEHU, N. PAVLOVSKY, E. C. TYLER-KABARA, S. CHASE, A. BATISTA;
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Abstract: Arm movements require a sensory-motor transformation. Sensory-motor transformations are studied using reference frame techniques. Researchers disassociate eye and hand position to reveal the spatial processing scheme used by reach-related cortical neurons. This disassociation between hand and eye position is a learned behavior, requiring extensive training. Neurons in the dorsal aspect of premotor cortex (PMd) have been variously reported to encode reach goals using a reference frame defined by the position of the hand alone, or by the positions of the eyes and the hand. The literature provides no consensus on how much of an effect the direction of gaze has on reach coding in PMd. We studied the effect of gaze on PMd cells during a memory-guided reach task with gaze unconstrained. We analyzed whether gaze influenced reach planning responses by using linear regression and neural prosthetic decode algorithms. In animals not trained to fixate, the neural encoding of reach goals in PMd is only weakly influenced by the direction of gaze. This confirms the findings of Cisek and Kalaska (Modest Gaze-Related Discharge Modulation in Monkey Dorsal Premotor Cortex During a Reaching

Task Performed With Free Fixation, 2002) but stands in stark contrast to the findings of Pesaran et al (Dorsal Premotor Neurons Encode the Relative Position of the Hand, Eye, and Goal during Reach Planning, 2006) and Batista et al (Reference Frames for Reach Planning in Macaque Dorsal Premotor Cortex, 2007). Our observations suggest that PMd neurons exhibit sensitivity to whatever task parameters contribute to rewarded behaviors.

Disclosures: B. Alemayehu: None. **N. Pavlovsky:** None. **E.C. Tyler-Kabara:** None. **S. Chase:** None. **A. Batista:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

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Topic: D.05. Visual Sensory-motor Processing

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Burroughs Welcome Fund Career Award

Howard Hughes Medical Institute

Champalimaud Neuroscience Programme

Fundacao para a Ciencia e Tecnologia

Title: Neural correlates of decision formation in PMd in a perceptual discrimination task

Authors: *D. PEIXOTO[1,2,3], R. KIANI[7], C. CHANDRASEKARAN[4], K. V. SHENOY[4,3,5,6], W. T. NEWSOME[3,8];
[1]Stanford Univ. Sch. of Med., Stanford, CA; [2]Champalimaud Neurosci. Programme, Lisbon, Portugal; [3]Neurobio., [4]Electrical Engin., [5]Program in Neurosci., [6]Bioengineering, Stanford Univ., Stanford, CA; [7]Ctr. for Neural Sci., New York Univ., New York, NY; [8]Stanford Univ. /HHMI, Stanford, CA

Abstract: Many prior studies of visually based decision-making have employed stimuli in which subjects decide the average direction of motion in stochastic random dot stimuli. Because subjects accumulate sensory evidence over time before committing to a decision, this framework provides an opportunity for electrophysiological analysis of evolving neural decision variables

that reflect integrated evidence. Most prior studies, however, have employed traditional single unit recording techniques, which limits their power to assess the dynamics of evidence accumulation and decision formation on single trials.
To address these issues, we recorded simultaneously from multiple units during the random dots motion discrimination task, using arm reach movements as the operant response. Neural activity was recorded from dorsal premotor (PMd) cortex, an area that is easily accessible to 'Utah' multielectrode arrays. Our goals were twofold: 1) to determine whether PMd activity reflects evidence accumulation toward decision about arm movement target selection, analogous to well-studied processes in pre-oculomotor structures, and 2) to leverage the statistical power conferred by simultaneous recordings to obtain insights into the decision formation process on single trials. We used logistic regression to predict the monkey's choices from neural population activity on individual trials using leave-one-out cross-validation. Logistic predictions were made in a 150 msec sliding window, allowing us to observe the evolution of decision-related activity in PMd. For strong motion coherences, average predictive activity, as measured by the fraction of correctly predicted trials, exceeded chance levels ~ 200 msec after onset of the visual stimulus, and reached 80% correct by 300 msec. As in LIP and other pre-oculomotor structures, predictive activity varied with stimulus coherence, rising faster and reaching higher levels for stronger coherences. Our data suggest that PMd activity reflects the accumulation of sensory evidence in the reach system, providing an opportunity to examine single trial neural dynamics underlying decision formation.

Disclosures: D. Peixoto: None. **R. Kiani:** None. **C. Chandrasekaran:** None. **K.V. Shenoy:** None. **W.T. Newsome:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

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Program#/Poster#: 263.14/QQ20

Topic: D.05. Visual Sensory-motor Processing

Support: Paul G. Allen Foundation

Title: Visual-motor processing in the central complex of walking *Drosophila*

Authors: *M. SIZEMORE, M. H. DICKINSON;
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Abstract: Successful navigation through a complex environment requires animals to extract useful sensory information and adjust their locomotor output accordingly, a process that involves

many stages. Whereas much work in systems neuroscience has focused on the initial and final steps of this process, relatively little is known about the critical bottleneck in which sensory signals are converted into a motor code. Investigating sensory-motor integration is particularly challenging, as it requires methods that permit recording and manipulating neurons and neural circuits while an animal is behaving. However, recent technical advances now make it possible to record from genetically-identified neurons in intact, behaving flies. We focus on the central complex (CX) as evidence from electrophysiology, anatomy, and behavior suggests that this region of the brain is a critical locus for visual-motor integration in flies and other insects.
As a first step towards exploring a visual-motor role for the CX during locomotion we have performed whole-cell current clamp electrophysiological recordings in flies walking on an air-supported spherical treadmill. We use machine vision to track the rotation of the sphere and provide visual stimuli using an arena of programmable LED panels. CX neurons are targeted by expressing GFP under control of the Gal4-UAS system. Cells are filled with biocytin during recording and visualized posthoc with confocal microscopy to determine cell morphology. We have recorded from fan-shaped body (FB) neurons and probed their responses to visual stimulation during walking and quiescence. While large field tangential neurons in the upper layer of the FB show spontaneous spiking activity that is unchanged whether the fly is walking or at rest, a class of tangential neurons in the lower layer of the FB increase their firing rate during bouts of walking. This increase can also be seen in the underlying membrane potential of neurons that are not spontaneously active, suggesting an increase in synaptic drive during locomotion. Drawing from available driver lines, we have also begun measuring the responses of classes of small field neurons in the fan-shaped body and other regions of the CX. By combining recording of individual classes of neurons with histological analysis of neuropil morphology we can begin to investigate sensory-motor processing in the CX.

Disclosures: M. Sizemore: None. **M.H. Dickinson:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 263.15/QQ21

Topic: D.05. Visual Sensory-motor Processing

Support: R01-EY017366

Title: Neuromuscular recruitment during oculomotor decision-making in human

Authors: *L. N. KATZ, J. L. YATES, L. K. CORMACK, A. C. HUK;
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Abstract: We sought to develop an experimental paradigm for use in humans that would provide a proxy for electrophysiological studies of oculomotor decision-making done in nonhuman primates. Neurons in the superior colliculus (SC) carry signals related to visual, cognitive, and motor components of eye-movements during decision-making. Unlike projections from SC to the oculomotor muscles, tectoreticulospinal projections from SC to the neck muscle system are not gated by omnipause neurons. This feature makes the neck a potentially fruitful site for probing oculomotor decision-making.

We recorded intramuscular electromyographic (EMG) activity bilaterally in a neck muscle involved in gaze shifts (splenius capitis, SPL) of 5 human subjects as they performed several variants of a well studied moving-dot direction-discrimination task, making either eye-only (head-fixed) or eye-and-head (head-free) gaze shifts to response targets. Gaze position was recorded using an Eyelink 1000 (500Hz, monocular, head free gaze tracking). EMG activity was recorded by inserting bipolar fine wire electrodes staggered 7-10mm apart along the axis of the muscle. We quantified responses by counting suprathreshold events or by rectifying and integrating the EMG signal.

Subjects performed both variable-duration (experimenter-controlled) and response-time (subject-controlled) versions of the moving-dot task. On each trial, subjects discriminated the direction of motion and conveyed their decision with a gaze shift to one of two targets (>30 degrees horizontal eccentricity). A burst of EMG activity was observed >100ms prior to saccade onset. In all head-free experiments, SPL was recruited during eye-and-head gaze shifts. In 19/28 experiments, SPL was recruited in a lateralized manner by eye-only gaze shifts made to targets at >15 degrees of eccentricity, in the absence of an overt head turn. In one-third of experiments (4/12), SPL activity during the response was modulated by stimulus coherence (task difficulty), with EMG amplitude inversely proportional to stimulus coherence. A similar effect on the latency of this response was observed in the response-time task (4/10 recording sites).

In conclusion, SPL EMG during oculomotor responses can be modulated by the strength of preceding sensory evidence. The richer and more prevalent effects seen in response-time conditions suggest that "leak" of SC activity may be more direct when decision formation and motor planning are coincident. Success in observing these cognitive signals in individual experiments is likely determined by differential innervation across electrode insertion sites and variable motor unit pooling.

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Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

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Program#/Poster#: 263.16/QQ22

Topic: D.05. Visual Sensory-motor Processing

Support: Marie Curie (EU)

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CIHR (Canada)

Title: MEG during planning of visually-guided pointing movements

Authors: H. ALIKHANIAN[1], *G. BLOHM[1], W. C. GAETZ[2], H. C. GOLTZ[2], J. F. X. DESOUZA[3], D. O. CHEYNE[2], J. CRAWFORD[3];
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Abstract: Planning visually guided reaches or pointing movements requires a complex sensory to motor transformation. Despite much recent progress, it still remains unclear where, how and when this transformation is carried out in the human brain. We use high spatio-temporal resolution magnetoencephalography (MEG) in an attempt to uncover (1) which brain areas are involved in transforming visual signals into appropriate motor commands for the arm, and (2) how does this transformation occur on a millisecond time scale.
Ten human subjects sat upright in the MEG apparatus and were asked to perform delayed (1500ms delay) visually-guided pro/anti-pointing movements towards (pro) or away from (anti) visual targets on a fronto-parallel screen. Pro- and anti-trials required opposite motor output following identical visual stimulation, which allowed distinction between the visual goal and motor plan. Subjects performed this task with either the left or right arm. To distinguish between intrinsic (muscle-based) and extrinsic (spatial) motor coding, we used three different forearm/wrist postures to perform the pointing with the right arm. A beamformer-based spatial filtering algorithm (event-related Synthetic Aperture Magnetometry) was employed to reconstruct brain activity from the MEG recordings. Brain areas were identified using a novel clustering method (Alikhanian, et al., 2013).
Preliminary analyses comparing differences in alpha and beta band synchronization for left versus right pro versus anti trials, show signatures of early coding of the visual goal (visual frame of reference) in V1-3, SPL, STS and IPL during the first 500ms after cue onset. At the same time, IPL, SPL and S1 also showed evidence for coding the motor plan, even at this early time after stimulus presentation. During the 500ms prior to movement onset, the early visual goal was coded largely present in V1-3, but there was also some evidence of visual information in

STS, FEF, PMd and ACC. However, there was now also a predominant motor code in IPL, S1, M1 PMv and even visual areas V2-3. We will also show effector and posture effects.
These findings suggest that the visual-to motor transformation occurs in a gradual fashion across the cortex and in time. In addition, visual/motor information seems to be coded in a frame of reference intermediate between the visual goal and the motor plan; indeed many brain areas show modulations both in a visual and motor reference frame. These findings are consistent with known monkey electrophysiology.

Disclosures: H. Alikhanian: None. **G. Blohm:** None. **W.C. Gaetz:** None. **D.O. Cheyne:** None. **H.C. Goltz:** None. **J.F.X. DeSouza:** None. **J. Crawford:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

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Topic: D.05. Visual Sensory-motor Processing

Support: HHMI

Title: Preparatory components and competitive advantages of Giant Fiber mediated escape takeoffs

Authors: *C. R. VON REYN, P. M. BREADS, G. M. CARD;
Janelia Farm Res. Campus, HHMI, Ashburn, VA

Abstract: Although escape behaviors are commonly associated with hard-wired, reflex-like motor programs, freely behaving animals perform complex and variable escapes when evading a predator. For example, as a simulated predator approaches, the fruit fly *Drosophila melanogaster* prepares for a jumping takeoff though a sequence of sub-behaviors, including freezing, postural adjustments, wing raising, leg extension, and wing depression. Using this sequence, the fly can alter both the trajectory and kinematics of its takeoff jump. However, the neural correlates orchestrating this behavior have yet to be identified. By recording whole-cell from tethered, behaving flies, we have demonstrated that two large descending neurons, the Giant Fibers (GFs), spike in response to looming stimuli mimicking approaching predators. Spiking in the GFs leads to a jump behavior, a rapid extension of the fly's middle legs. Here, we investigate how the GFs are incorporated into the sequence of sub-behaviors leading to an escape jump and what advantage is gained by enlisting the GFs in the behavior. Using whole-cell recordings and selective genetic silencing of GFs in freely behaving flies, we find that the GFs are involved in a subset of escape takeoffs. GF-mediated takeoffs require little preparation time, and often bypass

the full wing raising sequence. In addition, the interval between the start of jumping leg extension and the start of the first wing downstroke is consistently short, similar to what would be predicted by the motor outputs of the GF pathway. Although takeoffs can be evoked either with or without activation of the GFs across looming speeds, only GF-mediated takeoffs occur before predicted capture at high looming speeds. This suggests that the role of the GFs is to mediate takeoffs in response to the most rapid threats, demonstrating that *Drosophila* must coordinate multiple neural elements in order to select an appropriate motor program for predator evasion.

Disclosures: C.R. von Reyn: None. **P.M. Breads:** None. **G.M. Card:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: D.05. Visual Sensory-motor Processing

Support: Fondation de France

Title: Eye dominance influences triggering action and interhemispheric transfer time: A Behavioural and Electrophysiological study

Authors: *R. CHAUMILLON, J. BLOUIN, A. GUILLAUME;
Lab. De Neurosciences Cognitives, Marseille, France

Abstract: Our dominant eye (DE) is the one we unconsciously choose when performing a monocular task. Several studies revealed that the stimulation of this DE activates a larger cerebral network and with shorter
latency than the stimulation of the non DE (e.g *Shima et al. 2010*; Neuroreport 21(12), 817-21). Despite these results, the functions and behavioral consequences of this lateralization remain poorly understood. Here, through a Poffenberger paradigm we performed behavioural and electrophysiological measures to decipher the temporal impact of eye dominance on visuomotor transformation and on interhemispheric transfer time (IHTT) respectively. Firstly, by selecting participants according to their DE and handedness, and varying the side of the stimulated visual hemifield in a simple reaction time task, we examine the influence of the eye dominance in a sensori-motor task. We showed that the temporal impact of eye dominance strongly depended on whether the participants were right- or left-handed. In right-handers, reaction times (RT) were shorter for targets presented in the hemifield contralateral to the DE whereas in left-handers, RT of left hand was shorter only in participants with right DE, without hemifield difference.

Additionally, from the first use of the Poffenberger paradigm (1912), the idea is that, by comparing for a given responding hand reaction times for each visual hemifield, an estimation of the IHTT could be obtained. The present study demonstrates that this paradigm cannot lead to the correct estimation of the IHTT. In addition, it gives an explanation to the often reported IHTT negative values that otherwise appear illogical. Secondly, still in a Poffenberger paradigm, we used EEG recordings to more precisely evaluate the IHTT (eg *Rugg et al. 1984*; Neuropsychologia 22(2),215-25). Preliminary results in right-handers show a faster IHTT in subjects with right DE compared to those with left DE. In addition only right-handers with right DE show an asymmetry with a faster IHTT from right to left than from left to right. In sum, all these data converge to demonstrate a substantial impact of eye dominance on neural mechanisms involved in converting visual inputs into motor commands. These new findings highlights the need to consider the eye dominance that appears to be a hidden factor, in studies investigating the neural processes underlying visually-guided actions.

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Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

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Topic: D.05. Visual Sensory-motor Processing

Support: European Commission Grant Cogsystem FP7-250013

Title: Mirror neurons responding to inaction in the monkey ventral premotor cortex

Authors: *L. BONINI[1], M. MARANESI[1], A. LIVI[2], L. FOGASSI[2], G. RIZZOLATTI[1]; [1]Italian Inst. of Technol. - Brain Ctr. For Social and Motor Cognition, Parma, Italy; [2]Dept. of Neurosci., Univ. of Parma, Parma, Italy

Abstract: Mirror neurons (MNs) are a class of cells originally discovered in the ventral premotor area F5 of the macaque that discharge both during the execution and the observation of goal-directed motor acts. Human studies showed that areas belonging to the MNs system become active not only when subjects perform or observe a certain motor act, but also when they imagine an action performed by themselves or by another agent. However, no single neuron data are available on the possible involvement of MNs in motor imagery processes. Two monkeys were trained to execute a visuomotor task and to observe an experimenter performing the same task (observation task). Both tasks included two main conditions: one required the monkey to grasp a target object (action), the other to stay still for the entire duration of the trial(inaction). At the

beginning of the visuomotor task, the monkey had to maintain fixation in complete darkness. A cue sound instructed it either to grasp (high tone - action) or simply to fix (low tone - inaction) the subsequently presented target. After 800 ms a light switched on revealing one among three different graspable objects (target presentation). At the end of the sound (go/no-go signal), the monkey had to reach, grasp and pull the object (0.8 s) in case of action condition, or simply to fix it (1.2 s) during inaction condition. In the observation task the monkey had simply to fixate a spot of light in its extrapersonal space, while the experimenter performed the same conditions of the visuomotor task.We recorded 847 grasping neurons from area F5. Of them, 210 discharged also during action observation, but not during target presentation (MNs). Interestingly, part of these MNs became active during the observation task before the high tone ceased (go signal - action condition), anticipating the upcoming experimenter's action, while others discharged during the observation task even when the low tone ceased (no-go signal - inaction condition) and the experimenter remained still. Surprisingly, the activation profile of this latter class of MNs during action observation perfectly matched their activation profile during inaction condition. Importantly, this activation appeared specifically during the observation task, although cue signals and reward contingency were the same in the correspondent condition of the visuomotor task. These findings demonstrate that, in specific circumstances, MNs can activate even in the absence of visual information on other's action, suggesting that they could play a role in the internal generation of motor representations underlying motor imagery processes.

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Poster

263. **Sensorimotor Transformations: Physiology**

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Topic: D.05. Visual Sensory-motor Processing

Support: NSF FlexEBio IGERT (0654112)

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Title: Identification and single-trial characterization of visuomotor networks using electrocorticographic (ecog) activity

Authors: *W. G. COON[1,2], A. GUNDUZ[3], P. BRUNNER[1,4,5], B. PESARAN[6], G. SCHALK[1,2,4];
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Abstract: Humans can rapidly respond to visual stimuli. Previous studies have established the anatomical pathways that connect visual and motor cortices in animals, and recent imaging studies have begun to identify analogous tracts in humans. However, the spatiotemporal dynamics of cortical processes that translate visual input into motor output, as well as their functional significance (in particular with regards to variability in reaction time) remain to be characterized in full detail. A better understanding of the characteristics of the processes involved in visuomotor function and the neural basis for reaction time variability would expand existing neuroscientific understanding and may lead to new approaches to improve human performance. However, previous methodological limitations did not allow for characterization of such visuomotor networks at high spatial and temporal resolution, and/or in single trials.
In our study, we overcome these limitations by recording electrical signals directly from the surface of the brain (electrocorticography (ECoG)), which combine high spatial and temporal resolution with a high signal-to-noise ratio. We recorded ECoG signals from six human subjects while they performed a visuomotor response task. In this task, subjects were asked to respond with a button press as soon as they detected a change in a visual stimulus. To begin to characterize large-scale cortical processes, we used spectral estimates of gamma power (70 170 Hz; thought to reflect local cortical activation) to identify the network of locations that was activated by that task. We then applied a sensitive detection procedure to each of these locations to establish the timing of the onset of significant gamma activity modulation in single trials. Ongoing work is beginning to describe the characteristics of those networks, as well as their relevance to single-trial variance in reaction time in single trials. Specifically, we study the temporal variance of the activity onset times as well as cortical activation indexed by gamma power of each location in the identified networks, and how those characteristics co-vary with reaction time.
In summary, our study is beginning to provide the first detailed characterization of the spatiotemporal dynamics and functional significance of the largescale cortical processes that translate visual input into motor output. This work is setting the stage for the development of functional models of large-scale cortical processes by leveraging the power of single-trial analyses of brain activity and behavior.

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Poster

263. **Sensorimotor Transformations: Physiology**

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Topic: D.05. Visual Sensory-motor Processing

Support: FIRCA (NIH, USA)

ANCyT (Argentina)

Title: Direct sensorimotor matching rather than motor prediction appears to subserve activity in primary motor cortex during action observation

Authors: *N. GUEUGNEAU[1], S. MC CABE[2], J. I. VILLALTA[2], V. DELLA-MAGGIORE[2]; [1]Univ. De Buenos Aires, Ciudad De Buenos Aires – Argentina, Argentina; [2]Physiol. and Biophysics, Univ. De Buenos Aires, Ciudad De Buenos Aires, Argentina

Abstract: Despite the large consensus concerning the fundamental implication of the human motor cortex in action observation, the mechanism mediating the modulation of activity in the primary motor cortex (M1) remains a topic of debate. Does action observation necessarily imply predictive mechanisms based on internal (forward) models (Wolpert et. al. 2003) or does it rely on a frame-to-frame matching of the observed action onto its motor representation (Rizzolatti et. al. 2001)? Here we used Transcranial Magnetic Stimulation to assess the time course of corticospinal excitability (CSE) during the observation of unpredictable grasping actions. Fourteen healthy subjects observed grasping movements which final goal was unknown before movement onset, while motor evoked potentials (MEPs) were recorded at specific time points from the first dorsal interosseous (FDI) and the aductor digiti minimi(ADM) of their right hand. In order to assess precisely the temporal coupling between activity in M1 and the observed kinematics, CSE was recorded at 5 key time points during the observation of 3 grasping actions which kinematic features were very similar during the first third of the movement but differed drastically during the last two thirds. One action was directed to a large object, another action was corrected online, i.e. its goal switched from the large object to the small object, and a third action opened and closed unexpectedly before grasping the large object. We predicted that a mechanism based on direct matching would yield a temporal pattern of CSE finely tuned to the movement kinematics for the three actions, with no lag beyond sensorimotor processing. On the other hand, a mechanism based on forward models would result in longer latencies due to the occurrence of changes in movement kinematics that could not be predicted based solely on the motor plan for the large or the small object. Our results showed that CSE closely matched the movement kinematics for the FDI ($F(8,12)= 2.55$; $p=0.014$), but not for the ADM ($F(8,12)= 0.81$; $p=0.59$).MEPs lagged the observed action by 40-80 ms. The fact that MEP latency is ~25

ms and that 80-100 ms are necessary for visual information to influence an ongoing movement (Desmurget et. al., 2000) point to direct matching as the mechanism subserving motor resonance.

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Desmurget M, Grafton S (2000) Trends Cogn Sci 4(11):423-431.

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Poster

263. **Sensorimotor Transformations: Physiology**

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Topic: D.05. Visual Sensory-motor Processing

Support: DFG research grant (SCHE 1575/1-1)

Title: Network dynamics of spike-spike interactions within and between frontal and parietal cortex

Authors: *B. WELLNER[1], S. B. SUWAY[2], H. SCHERBERGER[1];
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Abstract: Recently, techniques to record many neurons in parallel have greatly improved. This development has shifted research from single electrode recordings toward the analyses of neuronal populations and their interactions that can be recorded from many electrodes in parallel. In particular, analysis methods of interactions of many single neurons and their network dynamics have not been well developed. So far, it is impossible to record from the whole brain network with cellular resolution. For a reasonably large dataset, we therefore focused on the parietal-frontal network for grasp movements of macaque monkey (area AIP and F5), which is well known for sensorimotor integration. Two monkeys performed a delayed grasping task consisting of a fixation period that is followed by a short cue, a memory period where the monkeys had to keep fixation, and a movement epoch where the monkeys had to grasp a handle with one of two grip types. In both animals 128 electrodes where implanted chronically in AIP and F5 (2 arrays with 32 electrodes per area). We recorded and analyzed single neuron activity during 10 recording sessions (monkey S: 6; monkey Z: 4) and isolated on average 77 single units per session (min 54; max 100) that were recorded in parallel. To reveal functional connectivity

between all single neurons we calculated crosscorrelograms (CCG) of all possible pairs and corrected for covariation in firing rates based on methods described in Smith and Kohn, 2008 and Ramalingam et al 2013. Our main interest was to characterize the network of all single neurons by applying complex network analysis described in Rubinov and Sporns, 2010. We were interested if our network was hierarchical and/or modular organized. As expected, different networks were active for the different grasp types. In both networks, motifs (groups of stronger interconnected neurons) were found that were comprised of a mixture of neurons from AIP and F5. We analyzed the degree of functional connections to see if there is a hierarchical organization. Results were power law-distributed indicating the presence of few highly connected neurons (hubs) in addition to a majority of only sparsely connected neurons. Hub neurons were mainly located in AIP and the power spectrum of their CCG with other neurons showed a strongly increased beta power. In contrast the CCG power spectrum of sparsely connected neurons exhibited no beta peak. These observations suggest that communication in parietal-frontal action networks has a small-world architecture with their hubs functionally connected predominantly in the beta frequency range.

Disclosures: B. Wellner: None. **S.B. Suway:** None. **H. Scherberger:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

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Program#/Poster#: 263.23/RR3

Topic: D.05. Visual Sensory-motor Processing

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Title: A coherent gate links memory and planning systems in macaque prefrontal cortex

Authors: *D. A. MARKOWITZ, B. PESARAN;
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Abstract: The lateral prefrontal cortex (LPFC) sits at the intersection of distributed brain systems that support working memory and behavioral planning. Patients with disorders of LPFC function, such as schizophrenia and ADHD, show deficits of memory-guided behavior, but often retain planning abilities in tasks where memory is not required. This suggests that an arrested flow of information from memory into the planning system in LPFC may contribute to behavioral dysfunction. To investigate this problem, we recorded from populations of neurons across multiple depths of LPFC in two Rhesus macaques as they performed memory- and visually-guided saccades. By comparing single unit activity during a delay period across these tasks, we find evidence for populations of neurons that encode Visual, Memory and Planning information, respectively. During correct trials, Memory unit spikes were significantly coherent with Planning unit fields in the 20-40 Hz band across these populations during memory delays ($p<0.05$, permutation test) but not during visual delays ($p\geq0.05$, permutation test), suggesting task-specific coupling between systems. By contrast, during memory saccade error trials, coherence between Memory and Planning systems was not significant ($p\geq0.05$, permutation test), and Planning but not Memory units exhibited significantly lower rates than observed during correct trials ($p<0.05$, permutation test). Viewed together, these task and performance dissociations indicate that coherent coupling could act as a gate that controls the flow of information between the Memory and Planning systems in LPFC.

Disclosures: D.A. Markowitz: None. **B. Pesaran:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

Location: Halls B-H

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Program#/Poster#: 263.24/RR4

Topic: D.05. Visual Sensory-motor Processing

Support: MEXT, Japan

Title: Distinct neuronal mechanisms for remembering multiple locations within vs. across visual hemifields

Authors: *A. MATSUSHIMA, M. TANAKA;
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Abstract: Humans can remember up to four visual items simultaneously (Luck and Vogel, 1997). Recent studies have demonstrated that this capacity limitation of working memory depends on spatial arrangement of items; performance is improved when visual stimuli are displayed across left and right visual hemifields, compared to when displayed within the same hemifield (Delvenne, 2005). Since most previous studies presented only a single visual cue to explore the neuronal correlates of spatial working memory, it remains elusive which mechanism attributes to the difference in memory capacity between the above two conditions.
We examined single neuron activities in the dorsolateral prefrontal cortex and adjacent frontal eye fields when monkeys remembered location(s) of one or two visual stimuli during the delay period. In the multiple-memory-guided saccade (MMS) task, two sample cues and three test stimuli were separately presented across a 2-s delay. One test stimulus was presented at the same location as one of the two samples (matched stimulus), while the others were presented elsewhere (non-matched stimulus). Monkeys were trained to maintain fixation on the central spot during the delay, then made a saccade to the matched stimulus upon the fixation point offset. Since the location of the matched stimulus was chosen randomly from the two sample locations, monkeys had to remember both locations. As a control, we also used the single-memory-guided saccade (SMS) task, in which one sample cue and three test stimuli were presented.
We compared the delay period activities between the tasks. When two samples were located across different visual hemifields ("Across" condition), neuronal activities were comparable to that in the SMS trials with a sample at the preferred location. On the other hand, when two samples were located within the same hemifield ("Within" condition), neuronal activity was the average of the responses in the SMS trials. To examine if these different neuronal representations might be reflected in behavior, we conducted an additional behavioral experiment with a test stimulus presented at the middle of the two samples. The rate of erroneous choice of the middle one was significantly higher in the Within than the Across condition, as predicted from the neuronal data.
These results suggest that the representations of items in different hemifileds could be maintained independently, while those of items within the same hemifield might interfere with each other, possibly through the lateral inhibition. These distinct neuronal representations might account for the different memory capacities between across- and within-hemifield conditions, reported in humans.

Disclosures: A. Matsushima: None. **M. Tanaka:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

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Title: Human intracranial recordings during spatial exploration of a virtual environment

Authors: J. SNIDER, *[1], O. J. AHMED, *[4], E. HALGREN[2,3], H. POIZNER[1,3], *S. S. CASH[5]; [1]Inst. for Neural Computation, [2]Dept. of Radiology, [3]Grad. Program in Neurosciences, UCSD, La Jolla, CA; [4]Dept. of Neurol., Massachusetts Gen. Hosp. & Harvard Med. Sch., Boston, MA; [5]Dept Neurol, Mass Genl Hosp, BOSTON, MA

Abstract: In humans, neuropsychological evidence supports a crucial role of the hippocampal formation and associated temporal lobe structures in forming and retrieving recent episodic memories, regardless of whether they invoke spatial processing. However, the role of the human hippocampus and temporal neocortex in spatial encoding remains unclear. We recorded local field potentials directly from dozens of cortical and subcortical regions in patients who were undergoing a diagnostic intra-cranial EEG (iEEG) procedure prior to surgical treatment for medication-resistant complex partial seizures. Two patients navigated a first person view about a virtual 'storage room' via a joystick. There were virtual objects placed on shelves in the room, some of which were covered by opaque bubbles. The subjects' task was to navigate to an indicated virtual bubble and pop it by aligning a virtual hand with the bubble and pressing a button. Popping the bubble revealed the object hidden underneath which then stayed visible for the rest of the block. Subjects then rated their interest in the object, high or low, before proceeding to the next bubble. During the task, subjects were either moving from bubble to bubble (walking) or standing still. Theta power (~6-8Hz) increased during walking phases in the hippocampus and perirhinal cortex. Beta power (~15-20Hz) over sensory motor cortex (one subject) increased while walking, potentially reflecting maintenance of the motor act of squeezing the joystick controller. Further, in a separate EEG experiment with a similar task, but active walking, we have seen spatial autocorrelations of subjects' EEG isolated to the low theta frequency (~2-8Hz) and localized over posterior parietal cortex (PPC). Intracranially, one subject with surface electrodes over PPC also showed increased activity in the ~3-8Hz range, but lacked the spatial autocorrelation of the PPC signal. Strikingly, intracranial recordings of theta from the anterior part of the left temporal lobe (present in both subjects) showed significant spatial autocorrelation with the subjects' simulated spatial location in the storage room. These findings suggest that, as in rodents, human anteroventral temporal lobe neuronal activity is related to allocentric space. This activity appears to extend to human cortical areas which do not have a clear homology in rodents.

Disclosures: J. Snider: None. **S.S. Cash:** None. **O.J. Ahmed:** None. **E. Halgren:** None. **H. Poizner:** None.

Poster

263. **Sensorimotor Transformations: Physiology**

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Support: MCYT SAF2009-10560

Junta Andalucía P09-CVI-4712

Title: Cortical dynamics during the preparation of antisaccadic and prosaccadic eye movements in humans in a gap paradigm

Authors: *M. ESCUDERO, I. CORDONES, C. M. GÓMEZ;
Univ. of Seville, Seville, Spain

Abstract: To compare the cortical dynamics of different oculomotor tasks, EEG and eye movements were recorded in 21 volunteers. Using a comprehensive approach, subjects were asked to perform saccadic tasks, which included a saccadic eye movement to a peripheral target (prosaccadic), a movement to the opposite side (antisaccadic), or maintain the gaze fixed (no-go). In mixed trials, prosaccadic, antisaccadic and no-go tasks were indicated by a color square (S1) present for 1900-2500 ms (instructive period). S1 disappeared for 370 ms (gap) and a black dot at 8 deg at right or left indicated the beginning of the task. Reaction times, amplitude of eye movements and number of errors were greatest in antisaccadic tasks, suggesting a greater difficulty. The EEG showed a contingent negativity variation (CNV) that increased progressively along the instructive period and suddenly during the gap: higher in antisaccadic, followed by prosaccadic and no-go tasks. Principal component analysis (PCA) disentangled fronto-central and occipital CNV-related and fronto-central gap-related components. The instructive period was characterized by fronto-central and occipital beta desynchronization (ERD) higher in antisaccadic than in no-go and parieto-occipital alpha synchronization higher in no-go than in antisaccadic tasks. During the gap, parieto-occipital beta and alpha ERD were higher in antisaccadic compared to no-go. The gap was further characterized by a fronto-central increase of inter-trial coherence in theta: highest during antisaccadic, followed by prosaccadic and no-go tasks. This phase locking in theta was also accompanied by theta ERS, which was significantly higher in antisaccadic than in the other two tasks. In PCA of spectral power two main components had dynamics similar to those extracted from voltage data, suggesting cross-

frequency coupling. These results suggest that the more difficult saccadic tasks are associated with top-down control mediated by frontal cortex, while simpler tasks rely more on bottom-up control mediated by posterior cortices.

Disclosures: M. Escudero: None. **I. Cordones:** None. **C.M. Gómez:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Topic: D.05. Visual Sensory-motor Processing

Support: Doctoral Student Research Grant, Competition #8, the City University of New York

Melvin Belsky Award in Biology, Brooklyn College of the City University of New York

Title: Behavioral responses to pulses of light in the Longfin Inshore squid

Authors: *S. P. HADJISOLOMOU, A. CHAVARGA, I. ABRAMOV;
Psychology, Brooklyn College, CUNY, Brooklyn, NY

Abstract: The unshelled coleoid cephalopods (octopus, squid, and cuttlefish) are renowned for their rapid, adaptive camouflage which is under direct neural control. This anti-predatory mechanism is extremely efficient at deceiving the visual system of predators and also allows for communication within and between species. This behavior is driven by a sensorimotor system, which receives information from the eyes and selectively activates intradermal color-pigmented skin cells called chromatophore organs. The question of how cephalopods control their chromatophores has received considerable attention from the perspectives of color modulation and contrast in ethology. While the anatomical arrangement of the neuro-muscular components of chromatophores and the sensory contributions of the visual system has been studied, the computation underlying the information processing of the chromatophore control system that enables such behavior is still unknown. The impulse-response system identification technique was used to test the hypothesis that a sudden, intense visual stimulus (pulse of light) can trigger chromatophore responses in squid. A camera recording at 240 Hz was used to capture behavioral responses before and after the pulse of light, providing a description of the timing relationships in the dynamics of brain function that control the chromatophore system response to light input. There was a rapid chromatophore expansion (~46 milliseconds) following the pulse of light. This behavior was extremely reliable across trials with a 3-second inter-stimulus interval (ISI), without signs of habituation. This is the first systematic study using the impulse-response system

identification technique to describe temporal dynamics of the squid sensorimotor system underlying chromatophore control. A description of the response to the flash illustrates the linear aspects of information processing in the squid brain and peripheral nervous system that control chromatophore activation across the skin.

Disclosures: S.P. Hadjisolomou: None. **A. Chavarga:** None. **I. Abramov:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

Location: Halls B-H

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Program#/Poster#: 264.02/RR8

Topic: D.05. Visual Sensory-motor Processing

Title: Rhythmical bimanual force production: Homologous and non-homologous muscles

Authors: *D. M. KENNEDY[1], C. WANG[2], J. B. BOYLE[2], C. H. SHEA[2];
[1]Dept. of Hlth. & Kinesiology, Texas A&M Univ., College Station, TX; [2]Hlth. & Kinesiology, Texas A&M, College Station, TX

Abstract: A large number of experiments over the last 40 years have isolated a coalition of constraints, including cortical and subcortical neural crosstalk, that influence the coordination of the two hands functioning together. Recent findings, however, have demonstrated that these constraints are eliminated or at least minimized when integrated feedback (e.g., Lissajous feedback) is used. An experiments was designed to determine participants' ability to coordinate 1:2 rhythmical bimanual force when homologous (N=10) and non-homologous (N=10) were activated. Given that neural crosstalk is defined as a mirror image command sent to the homologous muscles of the contralateral limb, we hypothesized that neural crosstalk should be more easily detected and characterized when the task required the activation of homologous muscles compared to when the task required the activation of non-homologous muscles to produce the goal pattern of coordination. The task was to rhythmically produce a pattern of isometric forces on a left side force transducer with the left arm that was coordinated with the pattern of isometric forces produced on a right sided force transducer with the right arm. The Lissajous display involved a goal template and a cursor indicating the forces produced with both limbs. The cursor moved from left to right as force was produced with the right arm and from bottom to top as force was produced by the right arm. The template illustrated the specific pattern of force requirements needed to produce the goal coordination pattern. The results indicated very effective temporal performance of the bimanual coordination patterns when both homologous and non-homologous muscles were activated. Additionally, the results indicated a

consistent and identifiable distortion in the force traces for both groups. However, it appears that crosstalk manifest differently during bimanual coordination depending upon whether homologous or non-homologous muscles are activated.

Disclosures: D.M. Kennedy: None. **C. Wang:** None. **J.B. Boyle:** None. **C.H. Shea:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Topic: D.05. Visual Sensory-motor Processing

Support: Deutsche Forschungsgemeinschaft DFG Su 693/1-1

Title: The allocation of attention during the acquisition of a novel visuo-motor transformation

Authors: *S. SUELZENBRUECK;
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Abstract: Our attention is generally focused on functionally relevant regions of our body and our environment ("selection-for-action", Allport 1987). Since for most actions our hands are functionally relevant, it is not surprising that increased attention is found for the area surrounding them. Increased attention is even found if the hands are not relevant for the respective task (Reed et al., 2006). When the segmental chain is extended by a tool, the location of the functionally relevant end effector is shifted from the hand to the tip of the tool. Previous studies indicate that during tool use increased attention is found for the area around the hand position as well as for the area surrounding the distal tip of the tool (Collins, Schicke & Röder, 2008; Reed, Betz, Garza & Roberts, 2010). For novel types of tools, so-called "pointing tools" (Holmes & Spence, 2005), the connection between the hand and the end effector is either only virtual (e.g. for the computer mouse) or cannot be accessed by direct vision (e.g. for the tools used in minimally-invasive surgical procedures). This experiment serves to investigate whether the same pattern of increased attention for the area surrounding the hand as well as for the area around the tip of the tool is also found for pointing tools and whether the locus of attention changes in the course of the acquisition of the visuo-motor transformation of the tool. Therefore the allocation of attention of two groups of young participants (n = 38) was investigated. Both groups were moving the effort arm of a two-sided lever to control a cursor on a computer monitor to different target positions. However, the groups differed in the visuo-motor transformations they had to master. While for one group the cursor represented the movements of the effort arm of the lever (a simple horizontal-vertical transformation), the cursor represented movements of the load arm of the

lever (an inversion of lateral hand movements and associated cursor movements). The distribution of attention was tested with a simple detection task which was preceded by the end of the goal-directed movement of the cursor to one of the targets. The endpoint of movement therefore served as an implicit cue for the subsequent detection task. The pattern of results indicates that indeed the distribution of spatial attention is modulated by the use of a pointing tool introducing a novel visuo-motor transformation, and that spatial attention changes in the course of the acquisition of a novel tool transformation. While attention is focussed on the cursor representing the tip of the tool at the beginning of practice, it is shifted towards the location of the hand position in later practice trials.

Disclosures: S. Suelzenbrueck: None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Topic: D.05. Visual Sensory-motor Processing

Support: MEXT, JAPAN 22120522 and 24120720

Title: Substitutional Reality for research in animal cognition

Authors: *Y. NAGASAKA, S. WAKISAKA, T. NOTOYA, N. FUJII;
RIKEN, Brain Sci. Institute, Lab. For Adaptive Intelligence, Saitama, Japan

Abstract: Comparative cognitive studies exploring dynamic social behavior have been accumulated. In the laboratory experiments, multiple trials under identical conditions are required, however, it has been difficult to present the same dynamic social situation in live. For human cognitive study, we recently have developed a substitutional reality (SR) system, an immersive human interface in which a participant were either presented with a live or a pre-recorded environment and hardly recognized which environment s/he was experiencing (Suzuki et al. 2012). In the present study, we modified the SR system for macaques (mSR) and evaluated it for practical use in the field of comparative cognition.
The mSR system consisted of a head-mounted display (HMD) fitted with a video camera at the front center of the HMD (mSR-headset), and a control PC. It had two presentation modes, 1) live-scene mode: the monkey viewed a live scene streamed from the camera, and 2) recorded-scene mode: the monkey viewed scenes that were pre-recorded by the camera. Because the monkey's head was calmly restrained, the scene in the headset was very similar in the live-scene and recorded-scene modes. In order to evaluate the system, we conducted a food-grabbing task in

which a monkey had to get a food from a stick handled by an experimenter (Chao et al. 2010). If the monkeys would react to a virtual environment in the same manner as in a live environment, this could validate the system for animal research.
In the experiment, the monkey wore the headset and performed the task under the live-scene mode after confirming successful performance in the task without the mSR-headset. All three monkeys performed the task successfully after a very short training (< 5 min) to get used to visual-motor-tactile coupling in the mSR system. Furthermore, under the recorded-scene mode all monkeys chased and tried to get the food; the behaviors were observed in the live-scene mode and the task without the headset. These observations suggesting that the monkeys experienced the virtual world with a feeling of being at a live event. In summary, we confirmed that the SR system was applicable for non-human animals, here macaques. The present mSR system is one of the ideal platforms to investigate cognitive processes in social interaction between macaques because of its capability for multiple presentation of the identical social situation many times, which generally occurs only once in the natural environment. Therefore, it opens the windows to broaden researches in comparative cognitive science.

Disclosures: Y. Nagasaka: None. **S. Wakisaka:** None. **T. Notoya:** None. **N. Fujii:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Topic: D.05. Visual Sensory-motor Processing

Support: Pioneer Grant 8 DP1 NS082121-02

Title: Chromatic properties of the phototactic response in the larval zebrafish

Authors: *D. A. GUGGIANA-NILO, C. RIEGLER, F. ENGERT;
Harvard Univ., Cambridge, MA

Abstract: Color vision research has made immense advances in determining the pathways followed by color information from the retina into the thalamus and then into the visual cortex. Although very comprehensive, these studies all result in a patchy and incomplete topographical map of the area being studied. This is a severe drawback when studying vision since it is known that the visual system operates in a number of parallel processing channels with a considerable degree of topography.
The zebrafish larva (Danio rerio) offers a unique platform to topographically characterize the chromatic component of the retinofugal projection, since its transparency coupled with the

ability to make transgenics expressing genetically encoded calcium indicators permits non-invasive 2-photon calcium imaging of many neurons simultaneously. Additionally the larval zebrafish retinofugal projections are all accessible and contain similar response properties and clustering as the mammalian visual system.
In this study we chromatically characterize the phototactic response in the larval zebrafish. The phototactic response is a well established and robust behavior in this organism and hence represents an ideal starting point to dissect the existing chromatic circuitry and its topology. Since the zebrafish has a tetrachromatic retina with UV sensitivity in addition to red, green and blue, we measured its 4-color behavioral spectral sensitivity function based on a previously reported phototaxis paradigm. In brief, we used a closed-loop projection system to present different colors at each side of the fish and force a choice of turning direction during each frame. The system allowed us to change light intensity and hue dynamically over the course of the experiment.
Our data supports previous observations of equivalent processing of all the color channels available to the fish, since turning direction preference depended solely on perceived intensity and not in the chromatic composition of the stimulus. In particular, preference peaked at a defined light level above background and then decreased all the way into avoidance when the light levels presented were very high.
As our next step the behavioral studies will then be complemented with functional imaging studies using multi-photon microscopy in combination with fluorescent protein-based calcium indicators. This will allow us to characterize the retinofugal projection in a color-dependent manner while the same visual stimuli used in the behavioral experiments are presented.

Disclosures: D.A. Guggiana-Nilo: None. **C. Riegler:** None. **F. Engert:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Professional Staff Congress (PSC)-CUNY Research Award Program

Title: Sociality predicts performance in exploratory behavior and sensorimotor gating in African cichlid fish

Authors: *T. PREUSS, M. PERKOWSKI, Z. BARANOV, L. DORDULAW, H. NEUMEISTER;
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Abstract: African cichlid fish (Astatotilapia burtoni) have a complex social hierarchy with males reversibly transitioning between dominant (DOM) and subordinate (SUB) phenotypes. DOMs exhibit frequent aggressive and territorial behaviors, threatening or attacking SUBs, which respond either proactively i.e., engage and flee, or reactively by avoiding interaction with DOMs altogether. Here we test the notion that males with a reactive copying style to threats will show anxiety-related behavior and adaptive changes in attentional information processing (i.e., sensorimotor gating, PPI). Focal observations of dominant and submissive behaviors over a two-month period in seven fish communities were used to distinguish three phenotypes, namely high, intermediate and low interactive males. Testing swimming activity (N= 31) in an open field paradigm revealed a significant ($p=0.05$) decrease of time spent in the center field (thigmotaxis) in low interactive (i.e., reactive) males compared to high and intermediate interactive (i.e., proactive) cohorts.
To test for PPI, subjects (N=33) were exposed to a startling auditory stimulus consisting of a sound pip (pulse), preceded by a non-startling sound pip (prepulse), and responses were scored based on the presence of a C-start startle response. Trials were conducted with two different prepulse/pulse interstimulus intervals (ISI 50 and 200 ms). Comparing subjects on the basis of social interactivity (low, medium, and high) revealed that low-interactive males exhibited a significantly ($p<0.05$) lower PPI rate than the two other groups for both ISIs.
Taken together, our results imply that the observed deficits in PPI in low interactive males are likely related to anxiety. More broadly, the identification of a PPI phenotype in cichlids may provide a novel opportunity to understand the neural basis of PPI since it uses natural psychosocial interactions instead of pharmacological means to modify PPI.

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Poster

264. **Sensorimotor Transformations: Behavior**

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ORF (Canada)

Title: Noise from stochastic reference frame transformations affects decision making

Authors: *T. MURDISON[1,2], H. LI[1,2], G. BLOHM[1,2];
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Abstract: It is known that reference frame transformations (RFT) induce variability in behavior and perception in sensorimotor tasks (McGuire and Sabes 2009; Burns and Blohm 2010; Burns et al. 2011). Thus RFTs must be regarded as being stochastic in nature. It is unclear whether RFTs also affect decision making, presumably though adding noise/variability to sensory evidence undergoing RFTs. Here, we investigated this effect using a 2-AFC random dot motion direction discrimination reaction time task with the head either straight or rolled to the left or right shoulder. Theory says that when the head is rolled, sensory signals need to undergo an RFT to rotate sensory signals into a reference frame appropriate for perception or action and we hypothesize that this will induce noise and deteriorate decision making. Four human subjects were asked to detect the direction (left or right) of a randomly selected subset of coherently (2, 10 or 20% coherence) moving dots. Subjects responded either with a left/right saccade or a button press. We created different RFT requirements by either (1) rotating only the on-screen stimulus, (2) rotating only subject's head, (3) rotating both the head and stimulus together or (4) rotating neither the head nor the stimulus.
Compared to no rotations (4), we found that head roll prolonged response latencies regardless of whether the retinal or spatial stimuli were rotated. This was true for both saccades and button press responses, with average increases (± SE) from 6.1% ± 9.3% to 19.9% ± 6.7% (corresponding to overall averages of 18 ms ± 85 ms and 110 ms ± 41 ms) for correct trials at the 10% coherence level. We also observed prolonged latencies with head roll across the other two coherence levels, with average changes from -1.2% ± 7.9% to 16.8% ± 6.6% (corresponding to -39 ms ± 73 ms and 84 ms ± 38 ms). Additionally, a standard LATER model for reaction times revealed that head roll induced variability into the rate of rise of the decision signal for all four subjects' saccades (increases from 58% ± 16% to 68% ± 10%) and for three of four subjects' button presses (increases from 86% ± 5.9% to 92% ± 3.2%) for correct trials at the 10% coherence level. These findings were representative of the other coherence levels.
Because using a button press response requires an explicit transformation of evidence into finger coordinates while saccadic responses technically do not require a RFT, our findings suggest that the sensory evidence used to make the decision is transformed into a common, non-retinal reference frame regardless of the response modality. These data show for the first time that reference frame transformations impact decision making.

Disclosures: T. Murdison: None. **H. Li:** None. **G. Blohm:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Topic: D.05. Visual Sensory-motor Processing

Support: Max Planck Society

DFG

Title: Influence of visual feedback on a goal-directed motor sequence: A virtual-reality study of prey capture in larval zebrafish

Authors: C. A. TRIVEDI[1], *J. H. BOLLMANN[2];
[1]Max Planck for Med. Res., Heidelberg, Germany; [2]Max-Planck for Med. Res., Heidelberg, Germany

Abstract: During goal-directed behaviors, the nervous system extracts relevant cues from the dynamic inflow of sensory information as a basis for selecting appropriate motor output. The motor output may be continuous or discrete in time depending on stimulus properties and the architecture of the nervous system. Prey capture in larval zebrafish is a visually driven goal-directed behavioral sequence in which target-directed swim bouts are separated by short periods of rest. These rest periods or inter-bout intervals (IBIs) range from a few tens of milliseconds to several hundreds of milliseconds and decrease monotonically with each subsequent swim bout as the larva approaches the prey. This burst and pause pattern, characteristic of intermittent locomotion, may enable the larva to sample visual input during the IBI and determine the subsequent motor pattern. Thus, testing the influence of individual stimulus properties on the IBI between chained swim bouts is of critical significance to begin to understand the neural mechanisms driving this goal-directed behavior.
In order to accomplish this, we reconstructed realistic stimulus sequences in a virtual reality assay based on a quantitative analysis of a wide range of stimulus conditions during naturally occurring prey capture. Minimally restrained larvae recapitulated target-directed swim sequences in a closed-loop projection system closely resembling natural prey capture behavior in terms of motor output parameters and monotonically decreasing IBIs. We then systematically varied the size, velocity, location and timing of the stimulus to test the input boundary conditions under which prey capture sequences could occur. Notably, the time course of these complex motor sequences was critically dependent on spatial and temporal properties of visual feedback. Having

characterized the influence of visual feedback on motor sequence generation, our virtual reality setup can be combined with functional techniques to enable the investigation of neural circuits involved in prey capture in larval zebrafish.

Disclosures: C.A. Trivedi: None. **J.H. Bollmann:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 264.09/RR15

Topic: D.05. Visual Sensory-motor Processing

Title: A shared representation of the space near oneself and another person in the human premotor cortex

Authors: *C. BROZZOLI, G. GENTILE, L. BERGOUIGNAN, H. H. EHRSSON; Karolinska Institutet, Stockholm, Sweden

Abstract: Interactions between people require shared high-level cognitive representations [1], but do people also share their representation of space? The human ventral premotor (PMv) and parietal cortices contain mirror neurons [2-4]. Here, we identified neuronal populations in the human PMv encoding the space near both one's own hand and another person's hand. We measured BOLD-adaptation [2] in 26 individuals to test whether neuronal populations showing selectivity to an object near one's own hand [5] also encode an object near another person's hand (Figure 1).

We compared BOLD-adaptation to a real 3D object appearing near another person's hand or a prosthetic hand following identical visual stimulation near the participant's own hand. By swapping the relative locations of the prosthetic and other person's hand (Figure 1c), we controlled for the unspecific effects of seeing a hand and the object moving in particular spatial positions in non-hand-centered coordinates.

The left PMv (inferior part of the precentral sulcus with the cluster encompassing the precentral gyrus; T=3.95, pFWE-corrected=0.015; Figure 1e) showed stronger BOLD-adaptation to the object presented near the real rather than the artificial hand following stimulation near the participant's own hand. Thus, the left PMv contains neuronal populations that encode the space both around the participant's and another person's hand.

In a second fMRI experiment (n=20), we tested for bidirectional adaptation [2]. The results showed that the left PMv adapts to the object near the participant's hand and the other person's hand independently of the order of presentation (T=3.77, pFWE-corrected=0.016; Figure 1f).

This finding indicates that the same neuronal populations in PMv encode peri-hand space both for self and other.

We identified a subpopulation of neurons in the left PMv with mirror properties. The shared premotor representation of peripersonal space could support social interactions by coding sensory events [6], action programs and cognitive processes [3-4, 7] in a common spatial reference frame for self and other.

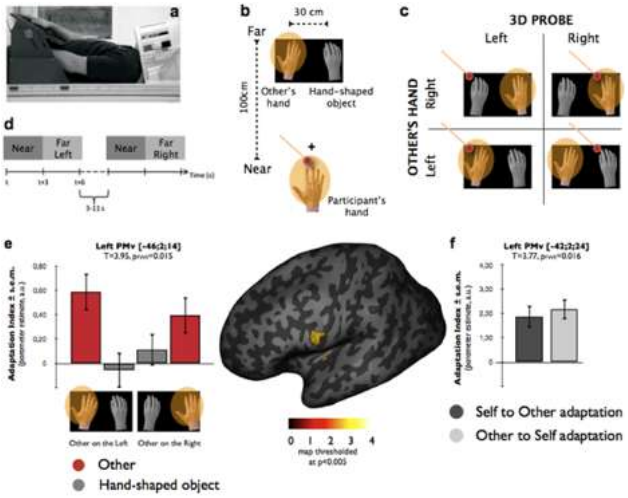

Figure 1. (a) Participant's tilted head and hand. (b) Spatial arrangement of the participant's, the other person's and the artificial hands around the fixation point (black cross). (c) Full-factorial design: independent manipulation of the positions of the prosthetic and the other's hand and the location of the 3D object. (d) Temporal schema of the events. (e) Experiment 1. A shared neuronal representation of the peri-hand space for self and other in the left PMv. The plots display the adaptation index. (f) Experiment 2. Significant bidirectional adaptation in the left PMv regardless of the order of stimulation.

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Disclosures: C. Brozzoli: None. **G. Gentile:** None. **L. Bergouignan:** None. **H.H. Ehrsson:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Topic: D.05. Visual Sensory-motor Processing

Support: BE, VK, and RS are supported by grants from NASA and the NSBRI.

SH and SM is primarily funded by the Velux Foundation.

Title: Motor cortex resting state functional connectivity in a large sample of healthy aging participants

Authors: *R. D. SEIDLER[1], B. ERDENIZ[2], V. KOPPELMANS[2], S. HIRSIGER[3], S. MERILLAT[3], L. JÄNCKE[3,4];
[2]Kinesiology, [1]Univ. Michigan, ANN ARBOR, MI; [3]Univ. of Zurich, Intl. Normal Aging and Plasticity Imaging Ctr. (INAPIC), Zurich, Switzerland; [4]Univ. of Zurich, Dept. of Psychology, Zurich, Switzerland

Abstract: Several studies have documented that aging is associated with impaired motor performance across a range of tasks, including decreased grip force strength, slowing of movement, and / or difficulties with balance and gait. Both primary neural representations of movement and potential compensatory cognitive mechanisms appear to be disrupted in older age (Seidler et al., 2010). Several studies have investigated age differences in the motor cortex during tasks that involve various motor paradigms, and one has investigated age effects on motor network connectivity (Langan et al. 2010), albeit with a relatively small sample size, and reported increased correlations between the two motor cortices for older adults. In the present study we investigated the effects of aging on functional connectivity of the motor network during the resting state in 212 healthy, right-handed subjects between the ages of 65 and 83. All participants had a normal mini-mental state exam (MMSE) (>26) and had no history of brain, heart, or metabolic diseases. The functional connectivity of the motor network in the resting state was measured by a seed-based correlation analysis. Based on a prior meta-analysis (Witt et al., 2008), a 4mm spherical region of interest (ROI) was defined in the left, dominant motor cortex. Participants' age and right hand motor tapping rates that were collected outside the scanner were used as covariates in a functional connectivity analysis. For this analysis we used the REST (Song et al., 2011) resting state functional imaging data processing software. Our results revealed that during the resting state, the left motor cortex showed correlations that varied with age and motor performance, primarily with the bilateral sensory-motor parts of the insular cortex and left middle frontal gyrus in the prefrontal cortex. Connectivity between the left motor cortex and these regions where higher in subjects that showed higher tapping rates outside the scanner. In addition, age was positively correlated with the strength of connectivity between left motor cortex and bilateral putamen. Collectively, the results of the current study showed that participants age is correlated with increased functional connectivity between the motor cortex and other cortical and limbic regions connectivity.

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Poster

264. **Sensorimotor Transformations: Behavior**

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Program#/Poster#: 264.11/RR17

Topic: D.05. Visual Sensory-motor Processing

Support: JST, CREST (JY210174).

JSPS KAKENHI (23300208)

Title: A motor control-learning model enabling stiffness adjustment according to uncertainty of object's weight during load-on task

Authors: *H. KAMBARA[1], H. OGAWA[1], D. SHIN[1], Y. KOIKE[1,2];
[1]Tokyo Inst. Technol., Yokohama, Japan; [2]Crest, Japan Sci. and Technol., Tokyo, Japan

Abstract: When we try to receive an object, our brain predicts weight of the object from its physical appearance. Motor command signals, according to the predicted weight, is then sent to muscles in advance of the object's placement on the hand. Prediction of external world dynamics and anticipatory control based on the prediction are the keys to generate stable movements actuated by the muscles of which force cannot be changed instantaneously. Previously, we had proposed a motor control-learning model for load-on task in which an object is placed on the hand (Kim et al., 2009). We had confirmed that the model can reproduce subject's motion if the correct dynamics, the weight of the object in this case, is given to the model as a context cue. In our real world, however, it is difficult to predict exact dynamics of the world. In case the dynamics cannot be predicted accurately, we often stiffen our body and prepare for unpredictable external force. When we ride a bike on a dirt road, for example, we often stiffen our arm to stable the handle against unexpected force disturbance exerted by stones on the road. In this study, we investigated whether our motor control-learning model can learn to adjust the stiffness of the arm according to the uncertainty of the prediction of object's weight.
We run computational simulations of load-on task in which actual object's weight is determined randomly with Gaussian distribution in each load-on trial but the predicted weight was kept constant in all trials at the mean of the distribution. As the results, we confirmed that stiffness level of the arm became higher as the variance of Gaussian distribution became larger. This result shows the ability of our model to adjust the stiffness level according to probabilistic features of the external world.

Disclosures: H. Kambara: None. **H. Ogawa:** None. **D. Shin:** None. **Y. Koike:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

Location: Halls B-H

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Program#/Poster#: 264.12/RR18

Topic: D.05. Visual Sensory-motor Processing

Support: ICORE

HFSP

Title: Sensory anticipation triggers activity in motor cortex

Authors: *D. REZNIK, R. MUKAMEL;
Tel-Aviv Univ., Tel-Aviv, Israel

Abstract: When we learn to execute a complex motor sequence (such as piano playing), we first break it into small fragments and then unify them to one seamless flow of actions. It has been suggested that mirror neurons might play a role in this dynamic coupling. Here we used whole-brain fMRI on 14 healthy right handed subjects to examine the role of the mirror system during anticipation of the sensory consequences of well learned motor sequences. The day prior to scanning, subjects were trained to perform 4 musical sequences on a digital piano keyboard - 2 unimanual sequences (one with the right hand and one with the left hand); and 2 bimanual sequences (one starting with the right hand and continuing with the left hand or vice versa). During the fMRI scan, subjects passively watched the same video recordings (including auditory feedback) that were used in the training session. We presented the subjects with full videos of the unimanual musical sequences and only the first half of the bimanual musical sequences (i.e., subjects saw the sequence played by one hand - thus creating expectation for the second half to be played with the other hand). We found that during the rest period following passive observation of the cropped bimanual videos, fMRI activity in primary motor cortex and in supplementary motor area was enhanced compared with the signal following viewing of the full unimanual videos. Interestingly, we found no lateralized response of this expectation signal in motor cortex - left and right motor cortices were activated to similar extent while expecting the sensory consequences produced by either hand. Our results support the involvement of the mirror neuron system during action preparation and expectation of musical sequences which is not specific to hand identity.

Disclosures: D. Reznik: None. **R. Mukamel:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Program#/Poster#: 264.13/RR19

Topic: D.05. Visual Sensory-motor Processing

Support: WT 087554

Title: Brain network properties of procedural consolidation

Authors: *S. SAMI[1], E. M. ROBERTSON[2], C. MIALL[1];
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Abstract: Consolidation of procedural memories is a time dependent process by which fragile short-term memories of skill are transformed into long-term stable ones. Current neuroimaging approaches highlight a shift in activity for some cortical areas with the passage of time following learning. However, many of the same cortical structures that are active in short-term memory are also active in long term consolidation. Questions regarding changes in the cortical networks that lead to increased functional stability remain unanswered. Here we address this question by investigating the global brain network properties before and after consolidation of two forms of learning in the serial reaction time task (SRTT), by sampling changes in neural connectivity via resting-state scans once before and at three time points after task acquisition. We show that learning under implicit and explicit conditions leads to similar graph network changes after a 6 hour period of wakeful consolidation. There was an initial decrease in betweenness centrality immediately after learning followed by a significant increase at 6 hours. These reciprocal changes are also evident for local efficiency measures i.e. an early increase in network efficiency is later reversed. This suggests that consolidation may therefore be characterized by a more redundant memory storage.

Disclosures: S. Sami: None. **E.M. Robertson:** None. **C. Miall:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

Location: Halls B-H

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Program#/Poster#: 264.14/RR20

Topic: D.05. Visual Sensory-motor Processing

Title: Sensorimotor adaptation is altered by mood priming

Authors: *O. L. BOCK[1], A. JESSE[2];
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Abstract: Cognitive functions are influenced by mood: happy persons show a preference for globalized and fast processing, while sad persons prefer localized and accurate processing. Here we investigated how mood might influence the functions involved in sensorimotor adaptation. Subjects pointed at circular targets arranged around a central starting point while vision of their hand was distorted by a 60 deg rotation. A cartoon face was briefly shown at target onset: a standard "smiley" in group S, a "frowny" in group F, and a neutral facial expression in group N. We found that the initial pointing error increased abruptly at the onset of the visual distortion and then gradually decayed towards a plateau. This decay was most pronounced in group F, less in group N, and even less in group S. Aftereffects upon removal of the distortion did not differ between groups. Since aftereffects are thought to reflect genuine sensorimotor recalibration while improvements during adaptation may also be due to workaround strategies, we conclude that recalibration is resistant to mood changes while strategies are not: smileys discouraged strategies, while frownys encouraged them. A preference for localized and accurate processing might be beneficial for strategic thinking.

Disclosures: O.L. Bock: None. **A. Jesse:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Topic: D.05. Visual Sensory-motor Processing

Support: NSF IGERT Grant DGE-0333451

NSF grants SBE 0542013

Title: Distributed action representations revealed with multi-voxel pattern analysis

Authors: *F. FILIMON[1], C. A. RIETH[2], M. I. SERENO[4], G. W. COTTRELL[3];
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Abstract: The roles of different brain areas in action representation and action understanding remain poorly understood. Based on neurophysiological evidence of mirror neurons in macaque ventral premotor area F5, the majority of human neuroimaging studies have emphasized the importance of Broca's area in action observation and execution, in addition to inferior parietal cortex and the STS. However, recent neuroimaging studies in both humans and macaques have revealed a much larger network of areas to be involved, outside of brain regions considered traditional mirror neuron areas.
We used multi-voxel pattern analysis (MVPA) to map the fine-grained representation of action execution, observation, and imagery in humans. Contrary to the presumed importance of traditional mirror neuron areas, we show that action representations:
1) are distributed across both dorsal (superior) and ventral (inferior) premotor and parietal areas;
2) can be discriminated in areas that are jointly activated by observation, execution, and imagery of reaching movements; and
3) can be accurately classified from either posterior parietal or frontal (premotor and inferior frontal) regions equally well.
These results challenge the presumed importance of traditional mirror neuron areas such as Broca's area in observation of action and action representation more generally. Moreover, unlike traditional univariate fMRI analyses, MVPA was able to discriminate between imagined and observed movements from previously indistinguishable BOLD activations in commonly activated regions, suggesting a finer-grained distributed pattern of activation within such areas.

Disclosures: F. Filimon: None. **C.A. Rieth:** None. **G.W. Cottrell:** None. **M.I. Sereno:** None.

Poster

264. **Sensorimotor Transformations: Behavior**

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Topic: D.05. Visual Sensory-motor Processing

Support: by EU FP7-FET project SpaceCog (600785)

Title: Updating target locations during continuous body motion

Authors: J. J. TRAMPER, *W. MEDENDORP;
Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Spatial updating is the process by which we keep track of objects in the space around us, even as we move our eyes, head or body. Many previous studies tested spatial updating *after*

an intervening movement, i.e., updating was probed in standstill. This work has shown that subjects make systematic errors in the updating process. Yet, many daily life situations require a continuous updating of the environment when we move. Therefore, in the present study, we examined the quality of spatial updating *during* ongoing motion. We subjected human participants to passive whole-body sinusoidal motion (A = 30 cm, f = 0.8 Hz), testing their updating behavior at different phases of the motion using a 2AFC psychophysical approach. After each reversal, when the chair reached a position of +15 cm or -15 cm, we briefly flashed an earth-stationary target light either in front or behind a fixation light. Then, after a displacement of 30 cm, we briefly flashed a probe light and subjects had to indicate whether they perceived the probe to left or to the right of the memorized target. Our null-hypothesis was that spatial updating during different phases of oscillatory self-motion is not different from each other, resulting in similar patterns of updating errors. Preliminary results show that during motion, subjects systematically erroneously update the location of remembered visual targets. We found that the updating errors were gaze-dependent, and that the direction and magnitude of the errors were similar to the results found for updating probed at the reversal points, i.e. in standstill. These results suggest that the integration of extraocular and vestibular signals to update a visual target is not affected by phase of the oscillatory movement.

Disclosures: J.J. Tramper: None. **W. Medendorp:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

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Topic: D.07. Vestibular

Support: Swiss National Science Foundation (PBZHP3-125519)

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Title: Continuous theta-burst stimulation of the right superior temporal gyrus impairs self-motion perception

Authors: *A. A. TARNUTZER, A. G. LASKER, D. S. ZEE;
Neurol., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Sensory input from the semicircular canals (SCC) and the otolith organs is centrally combined with signals from other sensory modalities to continuously update the internal estimate of self-motion. Constant-velocity vertical on-axis rotation leads to a decay of the nystagmus response from the horizontal SCC (angular vestibulo-ocular reflex) as well as of perceived angular velocity (PAV), and when the rotation stops a similar oppositely directed post-rotatory response occurs. Case reports and electrical stimulation studies suggest an involvement of the temporo-peri-Sylvian vestibular cortex in generating the PAV. Here we transiently inhibited parts of the temporo-peri-Sylvian vestibular cortex by use of continuous theta-burst stimulation (cTBS) and predicted an accelerated decay of PAV. Constant-velocity (100°/s) vertical-axis rotations were applied over 75 seconds before (1 block) and after (3 blocks) cTBS over the right superior temporal gyrus in six subjects. Breaks between the rotations (75sec) were initiated by abrupt stops. By use of a rotating potentiometer subjects indicated the PAV during and after the chair rotations. Simultaneously eye positions were recorded using a scleral search coil. Post-rotary PAV showed a trend towards shortened decay time-constants compared to the control trials ($p=0.086$) in the first 25 minutes after cTBS, while per-rotary PAV was not significantly changed. However, only early after cTBS the post-rotary PAV decay time-constant was significantly (9.4 ± 5.7 vs. 13.6 ± 5.9sec; $p=0.049$) reduced. Per-rotary and post-rotary angular vestibulo-ocular reflex decay time-constant were not significantly changed after cTBS ($p>0.05$). The findings from this pilot study support the hypothesis that the right temporo-peri-Sylvian vestibular cortex is involved in generating the percept of self-motion and can be modulated by means of cTBS.

Disclosures: A.A. Tarnutzer: None. **A.G. Lasker:** None. **D.S. Zee:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

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Topic: D.07. Vestibular

Support: CIHR

NIDCD grant R01-DC002390

Title: Characterization of central vestibular neuron responses during electrical stimulation delivered by a vestibular prosthesis

Authors: *D. E. MITCHELL[1], C. C. DELLA SANTINA[2], K. E. CULLEN[1];
[1]Dept. of Physiol., McGill Univ., Montreal, QC, Canada; [2]Dept. of Otolaryngology - Head & Neck Surgery, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Lesions that cause loss of vestibular function are relatively common in humans and hinder the ability to maintain stable gaze and balance. Ongoing research is focused on developing a chronically implanted vestibular prosthesis as a treatment option for patients suffering from vestibular loss. This device consists of a gyroscope, which senses the movement of the head, and electrode arrays implanted in each semicircular canal, which stimulate the vestibular nerve to send head motion information to the brainstem. Behavioural studies have shown that vestibular prosthetic stimulation produces reflexive eye and head movements, although these responses remain smaller than what would be expected from normal vestibular function. For example, the gain and time constant values of vestibulo-ocular reflex eye movements evoked by prosthesis stimulation are often lower than those observed in response to natural stimulation. To improve stimulation protocols and behavioural performance, it is necessary to determine how the brain responds to this type of stimulation.
Thus, to better understand the effect of prosthetic stimulation protocols on vestibular processing, we recorded the responses of neurons at the first stage of central processing (the vestibular nucleus) in alert rhesus monkeys while simultaneously delivering prosthetic stimulation. Neurons were identified based on their responses to eye position and during whole-body rotation. For neurons receiving direct input from the vestibular nerve, we determined the stimulation current amplitude for each unit by gradually increasing the current amplitude of biphasic pulses (200μs/phase) delivered at 100pps until spikes were reliably evoked at monosynaptic latencies (0.7-1.3ms). We ensured that the current amplitude used did not elicit any signs of facial nerve activation. Next, we applied pulse trains of 50pps-300pps to determine how neurons responded to sustained stimulation. We found that each neuron's spiking activity was highly time locked to pulses delivered through the prosthesis indicating that the vestibular afferent population was synchronously activated by prosthetic stimulation, which in turn drove synchronous central responses. We hypothesize that this synchronicity is in part the cause for the low behavioral performance obtained using the vestibular prosthesis thus far, and predict that desynchronizing afferent inputs will enhance the response of central vestibular neurons to prosthetic stimulation thereby improving behavioural performance. This work was supported by CIHR and NIDCD grant R01-DC002390.

Disclosures: D.E. Mitchell: None. **C.C. Della Santina:** None. **K.E. Cullen:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

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NCRR ITHS ignition award RR00166

Coulter Foundation

Cochlear, Ltd.

Sara Kranwinkle

Title: Human postural responses to stimulation of individual semicircular canal ampullae changes with head orientation

Authors: C. PHILLIPS, C. DEFRANCISCI, L. LING, K. NIE, A. NOWACK, *J. O. PHILLIPS, J. T. RUBINSTEIN;
Oto-HNS, Univ. of Washington, SEATTLE, WA

Abstract: Purpose: Vestibular sensory information is represented in a head centered coordinate frame. Changes in head position must be accounted for to produce appropriate postural responses to vestibular end organ inputs. We have developed a vestibular prosthesis for human use that targets individual ampullar nerves of the vestibular labyrinth. This allows us to evaluate individual semicircular canal contributions to the control of balance and posture in human subjects and their interaction with head position.
Methods: 4 patients with intractable unilateral Meniere's disease in the right ear were implanted with a vestibular prosthetic, employing three arrays of stimulating electrode sites inserted in the perilymphatic space adjacent to the ampulla of each semicircular canal. We tested the effects of electrical stimulation of individual canals on posture, as measured by computerized dynamic posturography. 2s duration 300 pulses/s trains of biphasic pulses of constant current were delivered during quiet stance in several postural contexts: stable support surface with eyes open and with eyes closed, with the subject's head oriented in one of seven positions: facing straight ahead, and turned horizontally 45º or 90º towards or away from the implanted ear, or pitched forward or back.
Results: Stimulation trains produced brief postural perturbations followed by a return to upright stance, the amplitude of which increased with stimulation current. With eyes open and eyes closed facing straight ahead, stimulation of the posterior canal produced an initial forward sway, while stimulation of the anterior canal produced an initial backward sway. Changes in head orientation during stimulation changed the amplitude and direction of the response. For example, during posterior canal stimulation, anterioposterior sway amplitude was greatly decreased with the head oriented away from the implanted ear and increased with the head oriented towards the implanted ear. In one subject, lateral canal stimulation produced a forward postural response

with the head oriented towards the implanted ear and a backward response with the head oriented away from the implanted ear.
Conclusion: Electrical stimulation of individual canals in human subjects produces consistent postural responses. The direction and amplitude of the postural response to stimulation in a specific canal changes with head orientation. These changes are consistent with canal-based models previously used to describe postural responses to transmastoid surface galvanic stimulation.

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Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 265.04/RR26

Topic: D.07. Vestibular

Support: NIH

CIHR

FQRNT

Title: Statistics of natural vestibular stimuli in monkey: Implications for coding

Authors: *A. D. SCHNEIDER, J. CARRIOT, M. J. CHACRON, K. E. CULLEN;
McGill Univ., Montreal, QC, Canada

Abstract: Understanding how neurons process sensory information requires not only a characterization of the neuronal responses, but also the natural stimuli encountered in an organisms sensory environment. This is in part because sensory coding is adapted to the statistics of natural inputs.

The vestibular system is comprised of 3 semicircular canals that sense angular velocity and 2 otolith organs that sense linear acceleration, in order to maintain stable gaze and posture. Canal afferents have been generally characterized by stimuli with standard deviations (std) around 40 deg/sec, and otolith afferents with linear accelerations of std around 0.2G (Fernandez and Goldberg 1971,1976). These neurons fire spontaneously around 100 Hz and are known to encode such stimuli through modulations in firing rate around the spontaneous value that are linearly related to the input, with precise spike timing not carrying behaviorally relevant information.

To characterize natural vestibular stimuli and their processing by the brain, we measured the head movements of Macaque monkeys, using a micro-electromechanical systems (MEMS) module sensitive to linear acceleration along and angular velocity about three axes (fore/aft, lateral, and vertical). Monkeys were free to move in a large play cage with a multi-level platform, while being monitored to identify behaviors associated with low (i.e. sitting and looking around), medium (i.e. foraging for food), and high (i.e. climbing and jumping across platform levels) levels of activity.

For the signals resulting from the three groups, we compared the probability distributions of the head angular velocity and linear acceleration signals to Gaussians with equal mean and variance, and found that the former have significantly longer tails as quantified by the fourth order moment kurtosis, as seen for natural auditory and visual stimuli (Ruderman 1994). We found that head angular velocity and linear acceleration signals could reach values up to 500-1500 deg/sec and 2-6G, respectively. Based on current otolith and canal afferent models, we predict that natural stimuli will elicit nonlinear responses in the form of cut-off (i.e. no firing) and saturation (i.e. firing at the maximum rate) in single afferents. We also predict that such nonlinear responses will synchronize the afferent population, in which case precise spike timing would carry essential stimulus information. These results challenge the traditional wisdom that early vestibular pathways are inherently linear, thus motivating a comprehensive rethinking of sensory coding in the vestibular system.

Disclosures: A.D. Schneider: None. **J. Carriot:** None. **M.J. Chacron:** None. **K.E. Cullen:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 265.05/SS1

Topic: D.07. Vestibular

Support: CIHR

Title: Impacts of neuronal sensitivity and variability on the encoding of linear self-motion

Authors: *M. JAMALI, J. CARRIOT, K. E. CULLEN;
Physiol., McGill Univ., Montreal, QC, Canada

Abstract: Understanding the neural code is a fundamental challenge in neuroscience. Of particular interest is the role of discharge variability in sensory encoding. Accordingly, we took advantage of the otolith system which is well-defined anatomically and physiologically and benefits from easily characterized sensory stimuli (i.e., head acceleration). Moreover, otolith afferents have a broad diversity in their spontaneous discharge regularity. Despite the heterogeneity in their background discharge, otolith afferents are known for their faithful encoding of linear head acceleration through firing rate modulation. Here, we employed information theoretic and gain measures to probe the impacts of background discharge regularity on the encoding of linear acceleration by primary otolith afferents.
Extracellular recordings were made from utricular afferent fibers in head-fixed alert macaques while seated in a primate chair and translated by a servomotor. Neuronal responses were recorded during i) sinusoidal as well as ii) random noise stimulation with a Gaussian distribution of head acceleration along each afferent's preferred direction within the behaviorally relevant frequency range (0 - 15 Hz) with a peak acceleration of 0.2 g (g = 9.8 m/s2). We found that while the sensitivity of both regular and irregular afferents increased monotonically as a function of sinusoidal stimulus frequency, sensitivities reached much higher values for irregular units. In response to noise stimulation, irregular units also conveyed ~2 times more information at higher frequencies (e.g., >6Hz), while in contrast regular afferents only transmitted slightly greater information at low acceleration frequencies (≤2Hz). Interestingly, the lower and upper bounds on the mutual information density had similar shapes as a function of frequency for either class of otolith afferents, indicating that a linear model of encoding was nearly optimal. Finally, we found the addition of spike timing jitter to afferents spike trains produced a significant reduction

in information transmitted by regular afferents whereas it had little effect on irregular units. Thus, compared to regular afferents and despite higher level of intrinsic noise in their spiking, irregular units on average convey more information about linear self-motion over a broad frequency range due to higher gains and require less precision in their spike timing to encode the stimuli. These results suggest that while highly sensitive irregular afferents are more advantageous for transient and dynamic stimuli, the regular units can provide accurate information when the stimulus is less dynamic (e.g. static tilt).

Disclosures: M. Jamali: None. **J. Carriot:** None. **K.E. Cullen:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 265.06/SS2

Topic: D.07. Vestibular

Support: DC04260

Title: Efferent control of spike rate variability in the peripheral vestibular system

Authors: *X. YU[1], J. D. DICKMAN[1], S. NEWLANDS[2], D. E. ANGELAKI[1];
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Abstract: Several studies have tested multiple hypotheses regarding the function of the vestibular efferent system, but little is currently known about how the efferent innervation of the vestibular periphery might modulate primary afferent activity. Sadeghi et al. (2007) specifically investigated a possible contribution of the efferent system to long-term processes of vestibular compensation after unilateral labyrinthectomy. Recordings from vestibular afferents revealed no difference in mean response properties, as compared to those in labyrinth-intact animals. However, response variability and detection thresholds were not compared.
Here we have measured direction detection thresholds of semicircular canal afferents using 0.5 Hz sinusoidal motion stimuli and ROC analysis. Canal afferent detection thresholds were measured from responses to 0.5 -16 °/s sinusoids delivered along the cell's preferred axis. Direction detection thresholds for semicircular canal afferents averaged 2.17°/s ±1.32 (geometric means± geometric deviation, n=85) for regular afferents and 1.91°/s ±1.58 (n=32) for irregular afferents in intact monkeys. After compensation from unilateral labyrinthectomy, canal afferent detection thresholds (from the contralateral vestibular nerve) were increased to 2.80°/s ±1.49 (n=66,$p<0.001$ comparison between intact and labyrinthectomy monkeys) for regular afferents

and 2.63°/s ±2.06 (n=29, p=0.12) for irregular afferents. Horizontal, anterior and posterior semicircular canal afferents had similar thresholds when tested along their preferred axis. Neuronal thresholds can depend on two properties that determine how much the two ROC distributions overlap: the difference in their means (amplitude slope) and their width (variance). No significant difference was found in the slope of response amplitude as a function of stimulus velocity for intact vs. labyrinthectomized monkeys. However, response variance was significantly increased after labyrinthectomy, and this increase lead to the increase in detection thresholds. Further analyses revealed that the increased variance had the timeframe of minutes (rather than seconds). The origins of such long-term control of neuronal variability, likely mediated by the vestibular efferent system, remain unknown. Further studies should also address whether the increased variances after labyrinthectomy were shared among the neurons or independent.

Disclosures: X. Yu: None. **J.D. Dickman:** None. **S. Newlands:** None. **D.E. Angelaki:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

Location: Halls B-H

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Program#/Poster#: 265.07/SS3

Topic: D.07. Vestibular

Support: NIDCD DC04260

Title: Vestibular behavioral thresholds for yaw rotation discrimination in rhesus macaques

Authors: *C. D. GARCIA, J. D. DICKMAN, D. E. ANGELAKI;
Baylor Col. of Medicine, Neurosci., Houston, TX

Abstract: The vestibular system provides for perception of movement, orientation in space, and motion related behaviors. In relation to self-motion perception, human yaw rotation velocity thresholds have been previously measured to be 0.7 deg s^{-1} at 0.5 Hz (Merfeld et al. 2008). However, the available data on neuronal direction detection thresholds for semicircular canal afferents in macaques have been quite disparate. For example, average neuronal direction detection thresholds for semicircular canal afferents were reported to be ~3.75 deg s^{-1} for regular afferents and ~8.25 deg s^{-1} for irregular afferents by Sadeghi et al. (2007). In contrast, Yu et al. found average direction detection thresholds for semicircular canal afferents to be much lower at 2.17 deg s^{-1} ± 1.32 (n=85) for regular afferents and 1.91 deg s^{-1} ± 1.49 (n=32) for irregular afferents (2013 SFN Abstract). Both studies used sinusoidal stimuli without simultaneously measuring behavioral thresholds. Furthermore, both studies compared neural thresholds in

macaques with human perceptual thresholds. Thus, it is possible that macaque and human perceptual thresholds are different. Here we set out to investigate how macaque perceptual yaw rotation velocity detection thresholds compare with human thresholds. We measured macaque thresholds using transient smooth trajectories that followed a 1s Gausian velocity profile (peak velocity: 1.3-30 deg s^{-1}). Two rhesus macaques were trained to perform a two-alternative-forced-choice task, in which they reported leftward or rightward rotation by making a saccade to one of two targets presented at motion end. Psychometric functions then determined direction detection thresholds over time. Mean yaw rotation detection thresholds were initially 8.59 deg s^{-1} for monkey H and 6.85 deg s^{-1} for monkey I. However, after continued training, perceptual thresholds in both monkeys decreased to 3.31 deg s^{-1} for monkey H and 1.48 deg s^{-1} for monkey I. In order to directly compare neuronal and perceptual detection thresholds for yaw rotation, we are currently measuring canal afferent thresholds simultaneously with perceptual detection thresholds in both animals.
This work was supported by NIDCD DC04260.

Disclosures: C.D. Garcia: None. **D.E. Angelaki:** None. **J.D. Dickman:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

Location: Halls B-H

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Topic: D.07. Vestibular

Support: NIH Grant EY12814

NIH Grant DC04260

Title: Reduced choice-related activity and noise correlations accompany deficits of perceptual and neural discrimination thresholds after unilateral vestibular lesion

Authors: *S. LIU[1], J. D. DICKMAN[1], S. D. NEWLANDS[2], G. C. DEANGELIS[3], D. E. ANGELAKI[1];
[1]Dept. of Neurosci., Baylor Col. of Med., Houston, TX; [2]Dept. of Otolaryngology, [3]Dept. of Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: Signals from the bilateral vestibular labyrinths work in tandem to generate robust estimates of our motion and orientation in the world. The relative contributions of each labyrinth to behavior, as well as how the brain recovers after unilateral peripheral damage, have been characterized for motor reflexes, but never for perceptual functions. We measured perceptual

deficits in a heading discrimination task following surgical ablation of the neurosensory epithelium in one labyrinth. We found large increases in heading discrimination thresholds and large perceptual biases at one-week post-lesion. Repeated testing thereafter improved heading perception, but vestibular discrimination thresholds remained elevated three months post-lesion. Electrophysiological recordings of single-unit activity from the contralateral vestibular (VN) and cerebellar nuclei (CN) revealed neuronal discrimination thresholds that were elevated as compared to normal animals, and trial-by-trial correlations with perceptual decisions ('choice probabilities', CPs) that were reduced. The relationship between CP and neuronal threshold was not significantly altered, suggesting that smaller CPs in lesioned animals were mostly attributable to greater neuronal thresholds.

Simultaneous recordings from pairs of neurons reveals highly structured correlated noise among subcortical vestibular neurons (Liu et al. 2013). In intact animals, although average noise correlations were close to zero, pairs of VN/CN neurons frequently exhibited large positive or negative noise correlations, and these showed a dependence on tuning similarity that was stronger than that typically seen for cortical neurons. Noise correlations are thought to arise from shared connectivity and common input. One of the sources of common input in the vestibular brainstem is convergence between the ipsilateral and contralateral labyrinths and this convergence might contribute to the large noise correlations observed in VN and CN (Liu et al. 2013). If so, a unilateral vestibular lesion should reduce correlated noise, in addition to reducing CPs.

Simultaneous recordings from pairs of neurons in lesioned animals revealed that the magnitude of noise correlations was systematically reduced after the lesion, along with the slope of the relationship between noise correlation and tuning similarity. Thus, cross-labyrinthine interactions appear to play important roles in enhancing neuronal and perceptual sensitivity, strengthening interneuronal correlations, and facilitating correlations between neural activity and perceptual decisions.

Disclosures: S. Liu: None. **J.D. Dickman:** None. **S.D. Newlands:** None. **G.C. DeAngelis:** None. **D.E. Angelaki:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

Location: Halls B-H

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Program#/Poster#: 265.09/SS5

Topic: D.07. Vestibular

Support: CIHR

Title: Neuronal ensemble coding in early vestibular pathways during self-motion

Authors: *A. DALE, J. CARRIOT, K. E. CULLEN;
Physiol., McGill Univ., Montreal, QC, Canada

Abstract: The secondary sensory neurons of the vestibular system receive inputs from vestibular afferents, and their outputs mediate postural reflexes as well as perception and computation of movement. Behavioral studies have revealed, however, that detection thresholds for perception are better than those of individual neurons (Seemungal et al. 2004, Grabherr et al. 2008). Recent work also demonstrated that pooling the activity of multiple neurons more closely matches the output of the vestibular nuclei (VN) with behavior (Massot et al. 2011). In order to accurately model the neuronal output, however, it is important to determine whether the responses of individual neurons are independent. Accordingly, we investigated noise correlations between the activity of 1) pairs of regular and irregular afferent inputs and 2) pairs of vestibular-only (VO) neurons in VN.
First we considered the baseline level of synchrony when no stimuli were present. We found that the magnitude of noise correlations for both afferent pairs and VO pairs was not significant. This revealed that in the absence of stimulation, neither afferent nor other putative common inputs synchronize the activity of neurons in VN. Next, we considered whether afferents or VN neurons displayed changes in synchrony during sensory stimulation. We found that during passive sinusoidal rotation, VO neurons fired more synchronous spikes than in the baseline condition, resulting in noise correlation magnitudes that were slightly elevated over baseline. In contrast, the magnitude of noise correlations between afferent pairs remained insignificant. While these results suggest that common afferent input influences synchrony between VN neurons more strongly during self-motion, they also reveal that the responses of individual VN neurons, like their afferent inputs, are largely independent.

Disclosures: A. Dale: None. **J. Carriot:** None. **K.E. Cullen:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

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Topic: D.07. Vestibular

Support: ERC Prop No: 283567

Title: Alpha-band modulations predict the quality of visual stability during whole-body motion

Authors: *T. P. GUTTELING, L. P. J. SELEN, W. P. MEDENDORP;
Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Despite the constantly changing visual input to the retina due to eye, head and body movements, we are able to maintain a stable representation of the environment. Various monkey single-unit and human imaging studies have suggested that the brain anticipates the visual reafference due to saccadic eye movement by remapping the neural image. A similar mechanism is thought to underlie visual stability during whole-body movements. Using electro-encephalography (EEG), we examined oscillatory activity related to spatial remapping during passive self-motion. Subjects (n=16) were seated in a computer controlled, custom-built vestibular sled, designed to impose translational motion along the interaural axis with a bell-shaped velocity profile (40cm, 1s). During the motion, subjects had to keep track of a remembered target that was initially presented in one visual hemifield, but needed to be remapped into the contralateral hemifield to maintain spatial constancy. The quality of the updating was characterized using a 2AFC task, in which we presented a second visual target and the subject had to indicate whether it was left or right of the original one. High-density EEG was recorded using an 88-channel active electrode system. Results show that the remapping of a spatial target during passive self-motion is reflected in lateralized alpha band (8-12Hz) activity. Alpha band suppression, indicative of enhanced sensitivity, occurs initially in parietal-occipital areas contralateral to the initial target location. During the motion, the shift of the remembered target to the opposing visual field was accompanied by a shift of alpha suppression to the other hemisphere. Importantly, the strength of the alpha band modulations correlated significantly with the updating error across subjects, i.e. a higher behavioral updating gain is associated with higher alpha band modulation. We conclude that self-motion remapping is reflected by alpha band activity, consistent with previous observation with head-fixed saccades. The role of other frequency bands in this process still remains to be studied.

Disclosures: T.P. Gutteling: None. **L.P.J. Selen:** None. **W.P. Medendorp:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

Location: Halls B-H

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Topic: D.07. Vestibular

Title: Modeling the influence of visual and vestibular tilt cues on the subjective visual vertical of rhesus monkeys

Authors: *M. F. KHAZALI, N. DADDAOUA, P. W. DICKE, P. THIER;
Cognitive Neurol., Hertie Inst. For Clin. Brain Res., Tübingen, Germany

Abstract: Our perception of the world as upright is reliably stable despite frequent changes of the orientation of the head and the body. The subjective visual vertical (SVV) is a measure of the percept of visual upright. The SVV of human subjects roll tilted relative to gravity shows deviations from the ideal. Actually, even without body tilt, the SVV can deviate from the gravitational vertical if a visual background (e.g. a visual frame), serving as reference, is tilted. The induced shift becomes more pronounced when the frame tilt is combined with body tilt.
In an earlier study (Daddaoua N. et al. , J. Vis., 8. 2008) we had tested the SVV of rhesus monkeys tilted around the roll axis in total darkness and found similar deviations as in previous work with humans. The human SVV has been successfully modeled by assuming that the SVV is an average of two vectors, namely a tilt estimate provided by the otolith organ and an idiotropic vector, corresponding to a representation of the body axis, needed to ameliorate insufficiencies of the otolith output for small tilts (Mittelstädt ,Naturwiss. 70, 1983). When applied to our monkey data, this model turned out to yield a good description of the experimental results obtained for monkeys as well.
In a subsequent experiment (Khazali M. et al., SFN2012 265.10) we had introduced a 28°x 28° visual frame tilted between +/- 45° in discrete steps around the vertical axis while the body axis of the monkey was upright or roll tilted by -45 or +45°. We found that the SVV was shifted relative to the vertical in the direction of the frame when the monkey was in the upright position. The amplitude of the induced shift was further increased when the monkey was roll tilted by +/- 45°. When again resorting to the Mittelstädt model (MM) to explain the experimental findings, we obtained reasonable fits, the exception being that the MM was unable to account for the increased amplitude of the shift of the SVV, observed when tilting both the frame and the body. To overcome this limitation we added features from another modeling approach (a Bayesian model proposed by Vingerhoets et al. , e.g. J. Neurophysiol. 101, 2009), namely the assumption that the vestibular signal is contaminated by noise increasing with tilt and secondly, the assumption that the visual background should be upright, a prior with tilt independent noise. Our model, combining the assumption of an idiotropic vector characterizing the MM, with key features of previous Bayesian approaches gives a very satisfactory description of the experimental findings, including the systematic variations known as A-and E-effects as well as the increased deviation of the SVV, observed when tilting both the body and the frame.

Disclosures: M.F. Khazali: None. **N. Daddaoua:** None. **P.W. Dicke:** None. **P. Thier:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: D.07. Vestibular

Support: NIH Grant DC04260

Title: Head direction-like cells in the primate subiculum and entorhinal cortex

Authors: *B. KIM[1], J. D. DICKMAN[2], J. S. TAUBE[3], D. E. ANGELAKI[2];
[1]Baylor Col. of Med., Katy, TX; [2]Neurosci., Baylor Col. of Med., Houston, TX; [3]Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Navigation is an essential skill for many animals and recent findings on place, head direction (HD) and grid cells in rodents provide a promising gateway to understand the neuronal mechanism of navigation. However few studies of the navigation circuit in primates have been reported. Here, we recorded the responses of cells in the subiculum (Sb) and the entorhinal cortex (EC), in rhesus macaques during 360° rotational motion in a dimly lit circular enclosure. The moneys had implanted search coils for monitoring eye and head position. The animals were trained to separate their head direction and eye direction by fixating separate visual targets. The animals' head was free to move within ±30° in the horizontal plane. Animals were trained to point a head-mounted laser onto a head target (green dot) and to visually fixate an eye target (red dot) that could either be coincident or separated from the head target. To determine directional tuning for each cell, we fit each neuron's response curves with von Mises functions in a head direction model and an eye direction model then compared the goodness-to-fit of each. As head direction and eye direction could be associated, we calculated the partial correlation coefficients and z-transformed them for comparison. Recordings from one monkey have revealed Sb and EC neurons that encode the monkey’s head direction relative to a visual cue card, similar to findings in rodents.

Disclosures: B. kim: None. **J.D. Dickman:** None. **J.S. Taube:** None. **D.E. Angelaki:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

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Topic: D.07. Vestibular

Support: NIH Grant DC012630

IPFW Faculty Summer Grant

Title: Head direction signal degradation impairs landmark navigation

Authors: S. L. KIRBY[1], R. E. HARVEY[1], E. A. GOEBEL[1], J. R. KOPPEN[2], D. G. WALLACE[2], *R. M. YODER[1];
[1]Dept. of Psychology, Indiana - Purdue U Fort Wayne, Fort Wayne, IN; [2]Dept. of Psychology, Northern Illinois Univ., Dekalb, IL

Abstract: Head direction cells provide a neural representation of perceived directional heading relative to the environment. Lesion studies suggest an important role for this signal in spatial learning and navigation, but brain lesions can damage additional brain structures/signals that may underlie the observed deficits. As a complementary model, we evaluated the spatial performance of otoconia-deficient *tilted* mice which have intact brains with degraded head direction cell signals. A caveat to this approach is that vestibular dysfunction can alter hippocampal function, and this change may impair spatial abilities. We therefore evaluated the integrity of hippocampal cholinergic afferents as a marker of hippocampal function. **Method:** Homozygous *tilted* mice and their heterozygous control littermates performed spatial tasks on two mazes: a 6-arm radial maze and a Barnes maze. Two tasks - an intramaze cue task and an extramaze cue task - were used on the radial arm maze, with the same two arms baited for four trials per day, across ten days. On the Barnes maze, mice performed four acquisition trials per day across four days with a constant goal location, followed by a single 5-min probe trial on the fifth day with no goal available. Optical density was measured in hippocampal sections stained for acetylcholinesterase. **Results:** *Radial arm maze* Control and *tilted* mice showed increased percentages of correct arm choices across days on both tasks. However, *tilted* mice were impaired on the extramaze cue task but not on the intramaze cue task, relative to control mice. *Barnes maze* Control and *tilted* mice showed decreased number of errors, distance, and latency across days. *Tilted* mice were initially impaired but performed similar to control mice by the last day of acquisition. Both groups spent similar amount of time in the target quadrant during the subsequent probe trial. *Optical density analysis* Markers of hippocampal cholinergic function were similar across control and *tilted* mice, suggesting the navigation deficits of *tilted* mice did not result from structural changes to hippocampus or to the cholinergic septohippocampal projections. **Importance:** These results support previous suggestions that the head direction signal is an important component of navigation relative to distal cues.

Disclosures: S.L. Kirby: None. **R.E. Harvey:** None. **E.A. Goebel:** None. **J.R. Koppen:** None. **D.G. Wallace:** None. **R.M. Yoder:** None.

Poster

265. **"Vestibular System: Sensory Coding, Perception, and Navigation"**

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Topic: D.07. Vestibular

Support: RFFI Grant 13-04-01736

Title: Short-term changes of hippocampal synaptic transmission after bilateral vestibular sensory loss in the rats

Authors: *J. N. ERON, V. A. KORSHUNOV;
Inst. of Higher Nervous Activity and Neurophysiol. of RAS, Moscow, Russian Federation

Abstract: Previously it has been shown in the number of human and animal studies that the damage of the vestibular sensory system causes the spatial memory deficit, the disorders in spatial perception and navigation and the reduction of efficacy of synaptic transmission and atrophy in the CA1 region of the hippocampus. The changes found after the complete vestibular loss (<60 days) were long-term. However, how the spatial information is encoded or compensated in hippocampus at the early stage after the complete loss or temporary deprivation of vestibular sensory inputs is unknown. It has recently been shown that the density of NMDA receptors was increased and no hippocampal atrophy was observed after the vestibular loss (Besnard et al., 2012).
The objective of this study was to estimate the efficacy of CA1 synaptic transmission in the early stage post-bilateral vestibular deafferentation (BVD). The fEPSPs of CA1 hippocampus were evoked by Schaffer collateral pair stimulation (at stimulus current ≤450 μA with interpulses interval 35 ms) and were recorded bilaterally in awaked freely moving rats (male Wistar rats, age 3-6 months). The animals were tested before and again 1, 2, 4, 5, 6, 10, 12, 15 days after BVD (n=3) or sham vestibular loss (n=2). There were no significant changes in the amplitudes for both fEPSPs in rats after sham surgery for post-operated term. In contrast, there was observed the typical dynamic change of fEPSPs during two weeks post-BVD term. After the BVD, the amplitudes of responses gradually decreased and achieved the maximal changes by day 4 (25-50%); however, by day 5 there was significant increase of the amplitudes of pair fEPSPs compared to controls. In 6 days after BVD the responses decreased again. The secondary facilitation of fEPSPs occurred during 10-12 days after BVD and then gradually irreversibly diminished over 15 days. Our data also showed that the decreasing of fEPSPs was accompanied by the increasing of threshold for synaptic transmission. The second of pair fEPSP had higher initial decrease and smaller its recovery than first one indicating the substantial derangement in

presynaptic transmission regulation.
Thus, two peaks in recovery of synaptic transmission efficacy in CA1 hippocampus after BVD was found in this pilot study. However, the mechanisms involved in the recoveries are still unknown. The facilitation could occur due to the local compensatory mechanisms of neuronal plasticity in hippocampus and also due to the external excitatory vestibular-independent interactions involved in spatial perception.

Disclosures: J.N. Eron: None. **V.A. Korshunov:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

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NIH Grant NS

Rita Allen Foundation

Title: Identification of Kv1.2 antisense RNA, an endogenous long non-coding RNA, in the dorsal root ganglion

Authors: *Y. TAO, X. ZHAO, F. E. ATIANJOH, J. ZHAO, L. LIANG, W. WANG, X. GUAN; Anesthesiol. and Critical Care Med., Johns Hopkins Univ. Sch. Med., BALTIMORE, MD

Abstract: Peripheral nerve injury down-regulates expression of Kv1.2 mRNA and protein in the dorsal root ganglion (DRG), a phenomenon that may contribute to induction of neuropathic pain. However, the molecular mechanisms that underlie this down-regulation are still unknown. Recent studies suggest that the mechanism for gene regulation involves widespread non- coding RNAs (ncRNAs), including long ncRNAs (lncRNAs). Here, we report a new native lncRNA that is expressed in the DRGs of rat, mouse, monkey, and human. Because most of its sequence is complementary to Kv1.2 RNA, we named it Kv1.2 antisense (AS) RNA. Kv1.2 AS RNA is expressed weakly in normal DRG. Approximately 21.5% of DRG neurons are labeled. Most were medium-sized, although some were small and a few large. Approximately 60.6% of Kv1.2 AS RNA-labeled neurons were positive for NF-200, 18.1% for P2X3, 15.3% for IB4, and 28.7% for CGRP. Although the distribution pattern of Kv1.2 AS RNA partially overlaps of Kv1.2 protein in DRG, most Kv1.2 AS-labeled neurons express a low level of Kv1.2 protein. The

characteristic of Kv1.2 AS RNA subpopulation distribution in the DRG indicates that it may have an important function in neuropathic pain.

Disclosures: Y. Tao: None. **X. Zhao:** None. **F.E. Atianjoh:** None. **J. Zhao:** None. **L. Liang:** None. **W. Wang:** None. **X. Guan:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Rita Allen Foundation

Title: Peripheral nerve injury up-regulates Kv1.2 antisense RNA expression in the injured dorsal root ganglion

Authors: *J. ZHAO, X. ZHAO, F. ATIANJOH, L. LIANG, W. WANG, X. GUAN, Y.-X. TAO;
Dept. of Anesthesiology/Critical Care Med., Johns Hopkins Univ., Baltimore, MD

Abstract: We recently identified an endogenous Kv1.2 antisense (AS) RNA in the dorsal root ganglion (DRG). We also found that this AS RNA specifically and selectively targeted Kv1.2 mRNA expression in vitro and in vivo DRG. It is known that peripheral nerve injury down-regulates expression of Kv1.2 mRNA and protein in the DRG. To investigate whether this down-regulation was due to Kv1.2 AS RNA, we examined expressional level of Kv1.2 AS RNA in the DRG after unilateral L5 spinal nerve ligation (SNL). The level of Kv1.2 AS RNA was time-dependently increased in the ipsilateral L5 DRG after SNL. Neither SNL nor sham surgery changed the expression of Kv1.2 AS RNA in the ipsilateral L4 DRG or L5 spinal cord. Furthermore, the staining density and number of Kv1.2 AS RNA-labeled neurons in the ipsilateral L5 DRG were higher than those in the contralateral L5 DRG on days 3, 7, and 14 post-SNL. These changes occurred predominantly in large DRG neurons. These observations were

confirmed in rats subjected to sciatic nerve axotomy. Our findings suggest that Kv1.2 AS RNA can be induced in the injured DRG after peripheral nerve injury. This induction may be responsible for DRG Kv1.2 down-regulation after nerve injury.

Disclosures: J. Zhao: None. **X. Zhao:** None. **F. Atianjoh:** None. **L. Liang:** None. **W. Wang:** None. **X. Guan:** None. **Y. Tao:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

Location: Halls B-H

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Program#/Poster#: 266.03/TT1

Topic: D.08. Pain

Support: NIH Grant NS072206

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NIH Grant da033390

Title: Impaired neuropathic pain and preserved acute pain in rats over-expressing voltage-gated potassium channel subunit Kv1.2 in primary afferent neurons

Authors: *L. FAN, J. ZHAO, V. TIWARI, Y.-X. TAO;
Johns Hopkins Univ. Sch. of Medicine/Accm, Baltimore, MD

Abstract: Voltage-gated potassium (Kv) channels are critical in controlling neuronal excitability and involved in the induction of neuropathic pain. Therefore, Kv channels might be potential targets for prevention and/or treatment of this disorder. We reported here that majority of the dorsal root ganglion (DRG) neurons were positive for Kv channel alpha subunit Kv1.2. Most of them were large and medium, although they had a verity of sizes. Peripheral nerve injury caused by lumbar (L)5 spinal nerve ligation (SNL) produced a time-dependent reduction in the number of Kv1.2-positive neurons in the ipsilateral L5 DRG, but not in the contralateral L5 DRG. Such reduction was also observed in the ipsilateral L5 DRG on day 7 after sciatic nerve axotomy. Over-expressing full-length Kv1.2 RNA in the injured L5 DRG attenuated the development of SNL-induced pain hypersensitivity without affecting acute pain and locomotor function. This effect might be attributed to the rescue of SNL-induced downregulation of Kv1.2 protein and the blocking of SNL-induced upregulation of endogenous Kv1.2 antisense RNA in the injured DRG. Our findings suggest that Kv1.2 may be a novel potential target for preventing and/or treating neuropathic pain.

Disclosures: L. Fan: None. **J. Zhao:** None. **V. Tiwari:** None. **Y. Tao:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Program#/Poster#: 266.04/TT2

Topic: D.08. Pain

Support: NS058886

NS072206

Title: Over-expression of dorsal root ganglion Kv1.2 AS RNA leads to neuropathic pain symptoms

Authors: *V. TIWARI[1], F. E. ATIANJOH[2], J. ZHAO[2], X. ZHAO[2], L. LIANG[2], Y.-X. TAO[2]; [1]The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Dept. of Anesthesiol. and Critical Care Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: We recently reported that peripheral nerve injury increased expression of an endogenous Kv1.2 antisense (AS) RNA in the injured dorsal root ganglion (DRG). To test whether this increase is involved in neuropathic pain development, we over-expressed Kv1.2 AS RNA in the DRG via injection of AAV5-Kv1.2 AS unilaterally into L4/5 DRGs. We found that expression of the Kv1.2 AS RNA was significantly increased in the $L_{4/5}$ DRGs at 4 weeks, reached a peak at 8 weeks, and remained high for at least 12 weeks after viral injection. In contrast, the expression of Kv1.2 mRNA and protein was significantly and temporally reduced in the ipsilateral $L_{4/5}$ DRGs. Behavioral observations showed that injection of AAV5-Kv1.2 AS, but not of AAV5-EGFP (as a control), produced mechanical and cold hypersensitivities as demonstrated by ipsilateral decreases in paw withdrawal threshold and paw withdrawal latency, respectively. These hypersensitivities developed by 4 to 6 weeks, reached a peak at 8 weeks, and were maintained for at least 12 weeks. Neither AAV5-Kv1.2 AS nor AAV5-EGFP affected locomotor functions. These findings suggest that Kv1.2 AS RNA-triggered DRG Kv1.2 downregulation induces mechanical and cold hypersensitivities, two major clinical symptoms of neuropathic pain.

Disclosures: V. Tiwari: None. **F.E. Atianjoh:** None. **J. Zhao:** None. **X. Zhao:** None. **L. Liang:** None. **Y. Tao:** None.

Poster

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Support: NIH Grant NS072206

NIH Grant NS058886

NIH Grant DA033390

Title: mTOR and its downstream pathway are activated in the dorsal root ganglion and spinal cord after peripheral inflammation, but not after nerve injury

Authors: *L. LIANG, B. TAO, L. FAN, M. YASTER, Y.-X. TAO;
Dept. of Anethesiology and Critical Care Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Protein translation controlled through activation of mammalian target of rapamycin (mTOR) participates in many physiological and pathological processes. However, whether such activation is required for chronic pain is still unknown. Here, we examined activation of the mTOR signaling pathway during complete Freund's adjuvant (CFA)-induced chronic inflammatory pain and L5 spinal nerve ligation (SNL)-induced neuropathic pain in rats. Western blot analysis showed significantly increased levels of phosphorylated mTOR (p-mTOR) and phosphorylated p70S6 kinase1 (p-S6K1, a downstream effector of mTOR) in the ipsilateral L4/5 spinal cord 2h,1 day,3days, and 7days after intraplantar CFA injection and in the ipsilateral L4/5 dorsal root ganglions (DRGs) 1 and 3 days after CFA injection. Immunohistochemistry also demonstrated increases in number of p-mTOR-labeled neurons in the ipsilateral L4/5 DRGs and in density of p-mTOR-labeled immunoreactivity in the ipsilateral L4/5 superficial dorsal horn 1 day after CFA injection. Moreover, intrathecal administration of rapamycin, a selective inhibitor of mTOR, significantly blocked CFA-induced mechanical allodynia and thermal hyperalgesia 1 day post-CFA injection. Interestingly, expression of neither p-mTOR nor p-S6K1 was markedly altered on days 3,7,or14 after L5 SNL in L5 spinal cord or DRG. These findings indicate that in DRG and spinal cord, mTOR and S6K1 are activated during chronic inflammatory pain, but not during neuropathic pain. Our results strongly suggest that mTOR and its downstream pathway contribute to the development of chronic inflammatory pain.

Disclosures: L. Liang: None. **Y. Tao:** None. **L. Fan:** None. **M. Yaster:** None. **B. Tao:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Support: NCATS 8UL1TR000149

NIH R01NS72890

R01DA019585

Title: The oxidized linoleic acid metabolite-cytochrome p450 system is active in biopsies from patients with inflammatory dental pain

Authors: *S. RUPAREL[1], K. HARGREAVES[1], M. ESKANDER[2], S. ROWAN[1], J. DE ALMEIDA[1], L. ROMAN[3], M. HENRY[1];
[1]Endodontics, [2]Pharmacol. and Endodontics, [3]Biochem., Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: Numerous studies have implicated a role for TRPV1 in mediating inflammatory hyperalgesia. Inflammation may lead to increased endogenous TRPV1 agonist activities. For example, oxidized linoleic acid metabolites (OLAMs) and the activity of oxidative enzymes releasing them [e.g., cytochrome P450 (CYP)], are upregulated following complete Freund's adjuvant (CFA)- induced inflammation in the rat. However, it is not known if such agonists are elevated in human inflammatory pain conditions. Since TRPV1 is expressed in human dental pulp nociceptors, we hypothesized that OLAM-CYP machinery is active in this tissue type and is increased under inflamed and painful conditions such as irreversible pulpitis (IP). Our data showed that the metabolism of exogenous linoleic acid (LA) was increased in IP tissues compared to normal tissues and pretreatment with a broad spectrum CYP inhibitor, ketoconazole, significantly inhibited LA metabolism. In addition, application of extracts obtained from LA-treated IP tissues onto cultured TG neurons, evoked significant inward currents that were blocked by pretreatment with the TRPV1 antagonist, IRTX. Moreover extracts obtained from tissues with LA and ketoconazole pretreatment showed significantly blunted inward currents in TG neurons. These data suggest that LA metabolites produced in human inflamed tissues act as TRPV1 agonists capable of activating peripheral nociceptors and that production of these metabolites can be targeted by inhibition of CYP enzymes. In addition, immunohistochemical analysis of two CYP isoforms, CYP2J2 and CYP3A1, were shown to be predominately expressed in immune cells infiltrating the dental pulp during inflammation, further emphasizing the paracrine role of CYP enzymes in OLAM-mediated inflammatory pain.

Disclosures: S. Ruparel: None. **K. Hargreaves:** None. **M. Eskander:** None. **S. Rowan:** None. **J. de Almeida:** None. **L. Roman:** None. **M. Henry:** None.

Poster

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R01NS72890

R01DA019585

Department of the Army W81XWH-07-1-0717

Title: Oxidative enzyme gene expression after traumatic injury

Authors: *H. M. BRANDFELLNER[1], S. B. RUPAREL[2], J. A. GELFOND[3], K. M. HARGREAVES[2];
[1]Gen. Surgery, [2]Endodontics, [3]Epidemiology & Biostatistics, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Adequate pain management in traumatically injured patients is difficult and often complicated by many adverse effects of the medications being prescribed. In response to injury and inflammation linoleic acid is metabolized to form oxidized-linoleic-acid-metabolites (OLAMs), which are direct agonists for the pain receptor, Transient Receptor Potential Vanilloid Subtype-1 (TRPV1). Thus, OLAM production after trauma likely contributes to pain. The list of possible enzyme candidates involved in metabolizing linoleic acid is large and before additional studies can begin to target those enzymes involved in OLAM formation, a narrower focus must be established. Here, we tested the hypothesis that traumatic injury triggers a sustained upregulation in the expression of mRNA transcripts encoding oxidative enzymes that may be involved in OLAM synthesis.
The Glue Grant Trauma-Related Database (TRDB) was used to identify changes in expression patterns of transcripts from peripheral circulating leukocytes (PCL) after traumatic injury. The Affymetrix HG-U133 Plus 2 human microarray was used to analyze a total of 105 genes, including 86 genes known to oxidize poly unsaturated fatty acids (the general class of lipids that

includes linoleic acid). 187 traumatically injured subjects had an associated 785 microarrays from PCL samples. The injured subjects were compared against 95 controls. Data was analyzed by log2 expression differences from control.
As compared to controls, traumatic injury in humans triggered significant upregulation of specific (Cytochrome P450 (CYP) and Lipoxygenase (LOX)) gene transcripts in peripheral circulating leukocytes samples. The most significant upregulated and down regulated genes with a $p<0.001$ were well conserved from Day 0 to 28 post injury. Analysis of the relationship between the genes and Injury Severity, Max Denver 2, and APACHE II scores demonstrated common overlap amongst the genes with $p<0.001$.
Traumatic injury triggers a massive, selective and sustained alteration in the expression pattern of enzymes capable of forming OLAMs in the blood. The CYP family appears to play a critical role in OLAM formation, thus identifying and targeting specific CYP enzymes responsible for OLAM synthesis after injury could provide potential strategies for development of novel analgesics.

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Supported by NIH 5T32GM079085, NCATS 8UL1TR000149, R01NS72890, R01DA019585, Owens Foundation, USAA Foundation President's Distinguished University Chair in Neurosciences (KMH), Department of the Army W81XWH-07-1-0717, and the Inflammation and the Host Response to Injury Large-Scale Collaborative Project Award 2U54GM062119.

Disclosures: H.M. Brandfellner: None. **S.B. Ruparel:** None. **J.A. Gelfond:** None. **K.M. Hargreaves:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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US Army W45MW30167N901

NCATS 8UL1TR000149

Title: Role of endogenous trpv1 agonists in a post-burn pain model of partial-thickness injury

Authors: *D. GREEN, S. RUPAREL, L. ROMAN, M. A. HENRY, K. M. HARGREAVES; Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: Background: Burns are an especially damaging form of trauma that few other injuries are capable of rivaling in regards to pain intractable to many available analgesics. The primary complaint of burn victims is an intense, often devastating spontaneous pain, with persistence of both mechanical and thermal allodynia and hyperalgesia.TRPV1 receptors are a class of ligand gated ion channels expressed on many pain-sensing nociceptive afferent neurons and transduce various noxious physical and chemical stimuli, including noxious heat (>48°C). However, the role of the TRPV1 receptor in mediating post-burn pain is incompletely understood. We previously have reported the production of endogenous TRPV1 agonists, oxidized linoleic acid metabolites (OLAMs), in skin heated to noxious temperatures. We hypothesize that persistent post-burn pain is mediated by a continuous release of membrane lipids from the burn site that activate or sensitize TRPV1.
Methods: Animals were anesthetized with isoflurane and a surgical plane of anesthesia was confirmed with a negative response to tail pinch. Thermal injury was induced by exposing an area of plantar hindpaw skin to a 100°C thermal stimulus for 30 sec. To promote reproducible thermal injuries, the same 1cm X 2cm region of the hindpaw was exposed in each animal, a stable stimulus temperature was maintained by a heating block and consistent hindpaw contact with the heated surface was achieved by placing a 30 g weight onto the dorsal hindpaw. Silver sulfadiazine cream (1%) was applied daily on the injured area to prevent infection. The injury was well tolerated and normal feeding and drinking behavior was maintained. OLAM levels in skin were obtained isolated after homogenization (Qiagen tissue lyser) in HBBS followed by extraction with 1ml chloroform:methanol (4:1; X1.5hr), addition of saline, centrifugation (335g X 5min) and drying the organic layer under N2. The lipid extract was re-suspended in 1ml of acetonitrile and subjected to LS-Mass Spectrometry to determine OLAM levels.
Results: The rat hind paw model of burn injury produced thermal allodynia peaking at 7d post thermal injury (ANOVA: $F=7.30$ $p<0.001$) and mechanical allodynia peaking at 14d (ANOVA: $F=55.61$ $p<0.01$). We observed that there was a significant ($p<0.05$) two-fold increase in OLAM levels from thermally injured skin when compared to extracts from skin obtained from non-injured animals.
Conclusion: These data indicate OLAMs are released from burned skin during times of post-burn allodynia and support the central hypothesis that oxidized linoleic acid metabolites contribute to persistent post-burn pain.

Disclosures: D. Green: None. **S. Ruparel:** None. **L. Roman:** None. **M.A. Henry:** None. **K.M. Hargreaves:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Title: Nerve growth factor-induced persistent allodynia is mediated by increased TRPV1 activities in rats

Authors: *M. ESKANDER[1], S. RUPAREL[1], E. POR[2], A. AKOPIAN[1,2], K. HARGREAVES[1,2]; [1]Endodontics, [2]Pharmacol., UTHSCSA, San Antonio, TX

Abstract: The management of pain remains a major health care problem due to an incomplete understanding of pain mechanisms, especially those involved in the transition to persistent/chronic pain states. Nerve growth factor (NGF) is a sufficient stimulus in humans to trigger a persistent pain state, but the mechanisms mediating this transition are unknown. We have tested the central hypothesis that NGF regulates the transition from acute pain to chronic pain via increased TRPV1 activities.
Using blinded observers, rats were injected with NGF for 5d and then tested for thermal allodynia in the hind paw up to 15 days after the last injection. NGF triggered a persistent thermal allodynia that extended up to 11d. Further, the allodynia was significantly reversed by systemic administration of anisomycin, a protein synthesis inhibitor ($p<0.01$) and by capsazepine (CPZ, $p<0.001$). Thus, NGF induction of a prolonged thermal pain state requires protein synthesis and is TRPV1-dependent. NGF treated animals also showed increased nocifensive responses to intraplantar capsaicin ($p<0.001$), suggesting either increased TRPV1 expression or sensitization. Therefore, we utilized whole cell patch clamp approaches to conduct a capsaicin concentration response in lumbar DRG cell bodies acutely cultured from NGF and vehicle treated rats. We observed a significant increase in Emax values from the NGF treated group with no significant difference in EC50 values. Western blot analysis indicated increased TRPV1 expression in lumbar dorsal root ganglia (DRG) plasma membrane after NGF treatment ($p<0.01$). Both electrophysiology and Western blot analysis indicate increased TRPV1 expression on the plasma membrane. Taken together, the results demonstrate that NGF produces prolonged state of thermal allodynia after systemic administration to rats that requires de novo protein synthesis and is mediated by increased TRPV1 expression. Further understanding of how NGF treatment modifies the TRPV1 channel and pore may help develop better analgesics for the treatment of chronic inflammatory pain.

Disclosures: M. Eskander: None. **S. Ruparel:** None. **E. Por:** None. **A. Akopian:** None. **K. Hargreaves:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Support: Wellcome Trust Strategic Award

Wellcome Trust Senior Investigator Award

Title: Investigating histone deacetylase four in peripheral sensory neurons

Authors: *M. CROW, S. B. MCMAHON;
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Abstract: Histone deacetylase inhibitors have been shown to be analgesic in a number of preclinical pain models, but the contribution of specific histone deacetylases (HDACs) to pain states remains unclear. HDAC4 has previously been linked to aberrant nociception, however the study of this gene with traditional knock out methods is limited by its developmental role in chondrocyte differentiation, which leads to early post-natal lethality. The aim of this project was to investigate the role of HDAC4 in sensory neuron function *in vivo* using the Cre-loxP system to conditionally delete HDAC4 from a subset of primary sensory neurons and characterise transcriptional and behavioural outcomes in naive animals and following nerve injury.
Conditional knock out (cKO) of HDAC4 from the Nav1.8-positive population of primarily small, nociceptive neurons did not cause overt morphological or sensory behavioral changes. QRT-PCR analysis of a number of pain-related mRNA targets in naive lumbar dorsal root ganglia (DRG) indicated that HDAC4 was dispensable for expression of these transcripts. HDAC4 expression was not required for mechanical hypersensitivity in the partial sciatic nerve ligation (PNL) model of neuropathic pain. However assessment of transcriptional changes 28 days after PNL revealed that HDAC4 cKO animals had significantly reduced levels of activating transcription factor 3 (Atf3) and neuropeptide Y (Npy) mRNA compared to littermate controls. Similarly, 24 hours after sciatic nerve transection, Atf3 mRNA levels were attenuated in HDAC4 cKO. As Atf3 is known to promote peripheral nerve regeneration, the sciatic crush model was used to test whether the reduction in Atf3 levels could negatively impact regeneration. HDAC4 cKO showed delayed sensory recovery but normal motor recovery, consistent with knock out in small fibres.
Loss of HDAC4 from primary sensory neurons results in aberrant transcriptional responses following axonal damage. Pilot data indicates that this may have consequences for peripheral

nerve regeneration, but not for nociceptive responses. HDAC inhibitors are broad-acting compounds; by understanding the role of individual histone deacetylases in sensory neuron function we will have the groundwork in place to develop and implement more targeted therapies for pain and nerve regeneration.

Disclosures: M. Crow: None. **S.B. McMahon:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: D.08. Pain

Support: Wellcome Trust

Title: Advancing the transcriptional profiling of dorsal root ganglia: A novel method to isolate pure neuronal subpopulations

Authors: *N. RICHARDS[1], M. THAKUR[1], S. MCMURRAY[2], S. B. MCMAHON[1];
[1]King's Col. London, London, United Kingdom; [2]Neusentis, Cambridge, United Kingdom

Abstract: Transcriptional profiling is a useful tool to help identify the molecular processes involved in normal physiological function, as well as in pathophysiological conditions. In recent years, ribonucleic acid (RNA) expression changes within the dorsal root ganglia (DRG) have been investigated extensively following peripheral nerve damage, with the aim of advancing our understanding of the mechanisms underlying neuropathic pain. However, the DRG is comprised of a heterogeneous population of cells, including sensory neuron cell bodies as well as many non-neuronal cells such as fibroblasts, glial cells and immune cells. To elucidate which transcripts are expressed in neurons alone, we have devised a novel technique to isolate sensory neurons whereby RNA can be successfully extracted and measured. This technique utilises fluorescence activated cell sorting (FACS) to obtain pure (>95%) neuronal populations from the rat DRG. Although the FACS process results in around a 75% loss of neurons, we have optimised labelling and RNA extraction to obtain high quality material (RNA integrity number >7) from cells fixed immediately following dissociation. We are now able to obtain enough RNA from these pure populations to measure transcripts of interest using microarray cards and next generation sequencing. This method is flexible and can be used to isolate various subpopulations of sensory neurons (e.g. A and C-fibre cell bodies, or peptidergic and non-peptidergic C-fibres). The application of this technique is likely to enhance our understanding of the specific contribution that sensory neurons make to chronic pain conditions.

Disclosures: N. Richards: None. **M. Thakur:** None. **S. McMurray:** A. Employment/Salary (full or part-time):; Pfizer Global Research and Development. **S.B. McMahon:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Support: ERC Paineuros 260435

Title: Transcriptional landscape of functionally distinct subsets of nociceptors

Authors: *A. REYNDERS[1], S. GAILLARD[1], S. NIEDELET[2], S. RIALLE[2], L. JOURNOT[2], A. MOQRICH[1];
[1]Ibdml-Umr6216 CNRS, Marseille Cedex9, France; [2]MGX (Montpellier Genomics), Montpellier, France

Abstract: Nociceptors belong to the neuro-sensory system and are the primary sensors of the pain pathways. Nociceptors have been shown to exhibit a tremendous molecular heterogeneity, which shapes their
physiological activity and their responsiveness to distinct pain qualities. Nevertheless, data regarding how gene expression is specifically regulated within molecularly-defined subsets of nociceptors, is notably missing. We have identified a novel protein, GINIP (Gα-Inhibitory INteracting Protein) expressed within a subset of non-peptidergic nociceptors and generated a knock-in mouse expressing the *m-cherry* at *Ginip* locus. By using this unique mouse model (*Ginip+/m-cherry*), in combination with an IB4 staining, we FACS-sorted three subsets of nociceptors for transcriptome-wide analysis: 1) $IB4^{+}GINIP^{+}$ -nonpeptidergic noxious mechanical transducers; 2) $IB4^{-}GINIP^{+}$ -non-peptidergic C-Low Threshold Mechano-Receptors 3) $IB4^{-}GINIP^{-}$nociceptors. The subsequent RNA-Seq deep sequencing experiment led to the generation of unique sets of data representative of pure transcriptome profiles of each subset. As a result of this pioneering approach, we established the combinatorial expression of the sets of genes that may dictate the functional specializations of considered nociceptors.

Disclosures: A. Reynders: None. **S. Gaillard:** None. **S. Niedelet:** None. **S. Rialle:** None. **L. Journot:** None. **A. Moqrich:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Program#/Poster#: 266.13/TT11

Topic: D.08. Pain

Title: Transcriptional and electrophysiological characterization of primary afferent human DRG neurons

Authors: A. GERLACH[1], J. THEILE[1], M. FULLER[1], K. YOGER[1], A. MOSS[2], B. SIDDERS[2], *M. L. CHAPMAN[1];
[1]Pfizer Neusentis, RTP, NC; [2]Pfizer Neusentis, Granta Park, Cambridge, United Kingdom

Abstract: The transmission of painful stimuli originates in a specialized subset of peripheral nociceptive neurons. Limiting the excitability of peripheral nociceptors is an attractive drug development strategy to intervene at the initiation of pain signaling and to minimize CNS-related dose limiting side effects. The cell bodies of most primary afferent nociceptive neurons are located in the dorsal root ganglia. Although the gene expression and electrophysiological profiles of nociceptors has been characterized to various extents in pre-clinical species, the relevance to human nociceptor function is not well demonstrated. We have used low-PMI human DRG tissue to examine the human DRG transcriptome and to examine the electrophysiological properties of viable cells.
Using RNA-seq on four unrelated human DRG we observe a correlation >0.9 between the transcriptome of the replicates compared to a correlation >0.95 between four inbred rats, suggesting that the variability between humans is not as great as often feared. Across 18,743 orthologous genes the overall correlation in gene expression levels is 0.6 between human and rat DRG, indicating a gross similarity between the species.The acutely isolated cells were found to have excitability properties similar to those reported in preclinical species. Application of 100 nM capsaicin resulted in a membrane depolarization (26 ± 5 mV, N=5/5 cells tested) and repetitive action potential firing, confirming the nociceptive phenotype of the cells. Resting membrane potentials were -66 mV to -27 mV in excitable cells (-45 ± 2 mV, N=40), with occasional spontaneous activity in the more depolarized cells. Voltage-gated sodium currents were too large to be accurately voltage-clamped in normal extracellular solution, requiring a reduction to 20 mM external Na^+. Under these conditions, TTX-resistant Na^+ current densities were 105 ± 26 pA/pF (N=13). Accurate quantification of fast TTX-sensitive currents was not feasible due to the size of the currents (>10 nA) and the size of the cells (76 ± 16 pF, N=6). In summary, low-PMI human peripheral nociceptive neurons are grossly similar to cells from preclinical species. Further biophysical and pharmacological characterization will be required to better determine the extent of cross-species similarity between human and rat nociceptive

neurons. A specific focus on the proteins differentially expressed in human and rat, the ion channels in particular, will likely uncover any subtle phenotypic differences not yet apparent.

Disclosures: **A. Gerlach:** A. Employment/Salary (full or part-time):; Pfizer. **M.L. Chapman:** A. Employment/Salary (full or part-time):; Pfizer, Neusentis. **J. Theile:** A. Employment/Salary (full or part-time):; Pfizer. **M. Fuller:** A. Employment/Salary (full or part-time):; Pfizer. **K. Yoger:** A. Employment/Salary (full or part-time):; Pfizer. **A. Moss:** A. Employment/Salary (full or part-time):; Pfizer. **B. Sidders:** A. Employment/Salary (full or part-time):; Pfizer.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Title: Identifying a population of peripheral neurons involved in mechanical hyperalgesia and allodynia in a mouse model of osteoarthritis

Authors: *L. D. TOWNSON[1], M. MINETT[2], J. WOOD[2], A. H. DICKENSON[1];
[1]NPP, [2]Wolfson Inst. for Biomed. Sci., UCL, London, United Kingdom

Abstract: AIMS: In order to elucidate new targets for analgesia in Osteoarthritis (OA), it is imperative that we understand the mechanisms underlying the chronic pain of this condition. Here we have tested the involvement of a specific population of peripheral neurons involved in the mechanical hyperalgesia and allodynia of OA using the Monosodium Iodoacetate (MIA) model of OA in genetically engineered mice.
METHODS: OA was induced using an injection of 0.2mg MIA in 5ul of 0.9% saline to the left knee of 6-8wk old anaesthetized mice in which all the post-mitotic sensory neurons containing sodium channel Nav1.8 had been eradicated through the expression of diphtheria toxin subunit A (DTA) (Science 321, 702-5). Behaviour was examined on days 0, 3, 10 and 14 to assess changes in the 50% paw withdrawal thresholds (PWT) and incapacitance scoring. This was followed by electrophysiological recordings from Lamina V WDR cells on days 14-16, where evoked responses to mechanical, thermal and electrical stimulation to the hind paw were recorded.
RESULTS: MIA produced a significant overall decrease in the 50% PWT of littermate controls, but not DTA mice, following induction of OA, demonstrating an essential role for Nav1.8-containing sensory neurons in mechanical hypersensitivity in OA. Channel transcripts enriched in Nav1.8+ neurons include TRPA1, the knockout of which has a Randall-Selitto loss of function phenotype in paw but not tail, and TRPC3 and TRPC6 that have been implicated in touch

transduction. Further analysis of channel knock-outs in the MIA model may help define molecular targets for OA treatment.
CONCLUSIONS: We demonstrate here that it is the population of Nav1.8 containing peripheral neurons that are responsible for the mechanical hyperalgesia and allodynia associated with OA. These neurons, previously established to have a substantial role in mechanosensation, cold and inflammatory hyperalgesia, could present a major target for future pharmacotherapy in OA.
We thank the Welcome Trust, MRC and Arthritis UK for generous support.

Disclosures: L.D. Townson: None. **M. Minett:** None. **J. Wood:** None. **A.H. Dickenson:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 266.15/UU1

Topic: D.08. Pain

Support: FAPESP

CNPq

Title: Role of nonpeptidergic subset of primary afferent neurons in inflammatory hypernociception in mice

Authors: *L. G. PINTO, G. R. SOUZA, A. H. P. LOPES, J. TALBOT, F. Q. CUNHA, T. M. CUNHA, S. H. FERREIRA;
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Abstract: Sensory information is transmitted from the periphery to the spinal cord by distinct subsets of primary afferent neurons, including small diameter unmyelinated C-fibers, which plays an important role in detecting noxious stimuli. C-fiber nociceptors have been divided into two classes, the peptidergic and nonpeptidergic. While many of the differences between peptidergic and nonpeptidergic neurons are now appreciated, a possible functional difference between these two classes of C fibers in the genesis of acute nociception as well as inflammatory pain is still unclear. Thus, this study aims to clarify the role of nonpeptidergic C fibers in acute nociception induced by mechanical, thermal and chemical stimuli as well as in inflammatory hypernociception. In order to elucidate differences between these two classes of C fibers, a neurotoxin was used to selectively eliminate the nonpeptidergic C fibers: a saporin conjugated to isolectin B4 (IB4). Nociceptive threshold was evaluated through thermal (Hargreaves) and mechanical (filaments and electronic von Frey) tests in C57BL/6 mice. Nociception models were

induced by intraplantar (i.pl.) injection of capsaicin and formalin (acute nociception) or by i.pl. administration of prostaglandin E2 (PGE2), epinephrine, endothelin, NGF, GDNF and carrageenan (inflammatory hypernociception). P2X3 and TRPV1 expression were analyzed by Western blot of dorsal root ganglion (DRG). The expression of IB4-labeled in spinal cord was determined by immunofluorescense using confocal microcopy. Firstly, it was observed that the intrathecal administration of IB4-saporin did not change baseline thermal and mechanical nociceptive threshold of the mice paw when compared to saline and saporin-control groups. The intrathecal administration of IB4-saporin reduced mechanical inflammatory hypernociception induced by carrageenan, epinephrine, endothelin, PGE2 or GDNF, but not NGF, in mice. Similarly, the treatment with IB4-saporin inhibited the nociception caused by intraplantar injection of the capsaicin. By contrast, the acute nociception induced by formalin did not change by administration of IB4-saporin. In addition, the expression of TRPV1 and P2X3 in DRG were reduced after treatment with IB4-saporin. Consistent with these findings, we found that IB4-saporin injection decreased the expression of IB4-labeled in spinal cord. These results suggest that absence the nonpeptidergic C fibers does not affect basal nociceptive threshold. However, these fibers are essential for the development of nociception in the paw of mice induced by inflammatory stimuli like PGE2, epinephrine, endothelin, carrageenan and capsaicin.

Disclosures: L.G. Pinto: None. **G.R. Souza:** None. **A.H.P. Lopes:** None. **J. Talbot:** None. **F.Q. Cunha:** None. **T.M. Cunha:** None. **S.H. Ferreira:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Support: NIH Grant R01 DE018025

NIH Grant P01 NS047472

Title: Genetic control of the segregation of sensory neurons innervating ectodermal versus mesodermal/endodermal tissues

Authors: *F.-C. YANG[1,2], T. TAN[1,2], T. HUANG[1,2], J. CHRISTIANSON[3,4], O. ABDEL SAMAD[1,2], Y. LIU[1,2], D. ROBERSON[2], B. M. DAVIS[3], Q. MA[1,2];
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Abstract: Sensory neurons of the dorsal root ganglion (DRG) receive painful stimuli throughout the body. The peripheral targets of DRG neurons can be divided into two zones: (1) superficial ectodermal tissues and (2) deep mesodermal/endodermal tissues. Here we identify a large molecular program that is required to selectively sense deep tissue pain. Retrograde labeling and genetic marking reveal that these genes are associated predominantly or exclusively in sensory neurons innervating deep tissues. Developmentally, expression of these genes is excluded from sensory neurons innervating superficial ectodermal tissues through Runx1-mediated repression. Accordingly, we show that the impairment of these deep tissue neurons in a mutant mouse model leads to the loss of sensory innervation to the deep tissues and the virtual abolition of muscle pain and visceral pain. Thus this study illustrates a genetic program controlling the molecular segregation of sensory neurons innervating ectodermal versus medodermal/endodermal tissues, and provides new insight into the molecular and cellular basis of deep tissue pain.

Disclosures: F. Yang: None. **T. Tan:** None. **T. Huang:** None. **J. Christianson:** None. **O. Abdel Samad:** None. **Y. Liu:** None. **D. Roberson:** None. **B.M. Davis:** None. **Q. Ma:** None.

Poster

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Topic: D.08. Pain

Support: NIH CA080153

Title: Contralateral tactile allodynia from ipsilateral ET-1 injection requires contralateral efferent nerve conduction

Authors: *G. R. STRICHARTZ[1], Y.-W. CHEN[2], J. C.-F. WANG[3], A. KHODOROVA[3]; [1]Dept Anesthesia Res. Labs, Pain Res. Center/ BWH, BOSTON, MA; [2]Physical Therapy, China Med. Univ., Taichung, Taiwan; [3]Pain Res. Ctr., Brigham & Women's Hosp., Boston, MA

Abstract: We previously reported that 1d after endothelin-1 (ET-1, 2nmol) is injected into one rat hindpaw, the contralateral hindpaw showed a heightened response to noxious mechanical and chemical (ET-1, formalin) stimuli. This sensitization was prevented by blocking afferent transmission with local anesthetic (0.25% bupivacaine) injected at the ipsilateral sciatic nerve before ET-1's injection into the ipsilateral paw. Current studies, presented at this SfN meeting,

report the essential role of spinal aPKC/PKMζ and PI3K in this same contralateral sensitization, confirming a role for central (spinal) sensitization in this process. In order to determine if an efferent outflow to the contralateral paw contributed to this contralateral tactile allodynia, we conducted the following experiments. After injection of ET-1 (2nmol), withdrawal thresholds of the ipsilateral (ILP) paw fell from 12g at baseline to 2g at 2.5h, 3g at 24h and 3g at 48h. The contralateral paw (CLP) threshold fell insignificantly at 2h after ET-1, but at 24h was significantly lower, at 4g. Direct injection of plain bupivacaine (0.25%, 0.04mL) into the plantar aspect of the ILP before ipsilateral ET-1 injection, causing analgesia to tactile stimulation for ~30 min, prevented the contralateral allodynia, without preventing ipsilateral sensitization, thus showing that local sensitization did not require the acute impulse activity necessary for CLP sensitization. This *Control* CLP sensitization was altered, however, when the *contralateral* sciatic nerve was blocked for many hours by a biodegradable microsphere formulation that slowly released bupivacaine (MS-BUP, 5mg). Injected 3h before the ipsilateral injection of ET-1, this dose of MS-BUP, which inhibited nocifensive responses to tactile stimulation in normal rats for 12h, prevented any significant drop in the CLP threshold from ipsilateral ET-1. Allodynia of the ILP was unaffected by this contralateral nerve block. These results show that efferent transmission through the contralateral innervation is necessary for sensitization of the CLP, and suggest that release of substances that cause paw sensitization in the CLP is essential for contralateral sensitization.
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Disclosures: G.R. Strichartz: None. **Y. Chen:** None. **J.C. Wang:** None. **A. Khodorova:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Support: NIH CA080153

Title: Evidence for spinal atypical PKCs and PI3K involvement in peripheral ET-1's induction of central sensitization

Authors: *A. KHODOROVA[1], G. STRICHARTZ[2];
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Abstract: Previously we have shown that the unilateral intraplantar (i.pl.) injection of ET-1 sensitized both the ipsilateral and contralateral paws to subsequent mechanical and chemical stimulation. For example, a second i.pl. dose of ET-1 (2 nmol), injected into the contralateral paw 24h after the first injection (also 2nmol), resulted in a greater acute nocifensive flinching response, as also occurred for the second phase of the response to contralaterally injected formalin after ipsilateral ET-1. Block of the ipsilateral sciatic nerve by local anesthetics during the initial ET-1 flinching period (~2h) prevented the later contralateral increase in acute nociception, confirming the importance of afferent transmission for contralateral hypersensitivity, and implicating central sensitization (CS) in these contralateral changes. However, the cellular mechanisms that maintain ET-1-dependent CS remain unclear. Because other peripheral substances that cause prolonged mechano-hypersensitivity, e.g. NGF, rely on spinal atypical PKCs (aPKCs), in the current experiments a myristolated pseudosubstrate inhibitor of aPKCs (PKC_{ζ}/PKM_{ζ}), mPSI, was injected intrathecally (i.t., 5g/10µl) 2 d before a first i.pl. ET-1 injection. Controls (i.t. vehicle) showed clear contralateral potentiation of the *acute nocifensive response* (TF = total number of hindpaw flinches/70min) to ET-1 (2nmol) (TF^{2nd}=225±38 compared to TF^{1st}=133±18 ; n=10, P<0.05).The inhibitor of aPKCs had no effect on the initial acute nocifensive response to ET-1 (TF^{1st}=129±31, n=10, p>0.5 compared to control, above), but fully prevented, even depressed, the contralateral sensitization (TF^{2nd}=87±18; n=10, P<0.05). In addition, i.t. wortmannin (5µg), a potent and selective inhibitor of PI3K, given 0.5 - 2h before the first ET-1 injection, did not affect the initial acute flinching, but fully prevented delayed sensitization. Peripheral mPSI (40-200µg/20µl), injected into the plantar paw a day before ET-1 (0.1 nmol), slightly attenuated the ipsilateral reduction of paw withdrawal threshold, by ~30% (dose 200µg; n=10), whereas local wortmannin (9µg total dose/per paw), was ineffective (n=4). The findings indicate that $aPKC_{\zeta}$/$PKCM_{\zeta}$ and PI3K at central loci are key mediators in the development of contralateral sensitization caused by subcutaneously delivered ET-1, while at the periphery $aPKC_{\zeta}$/$PKCM_{\zeta}$ makes a minor contribution, and PIK3K none, to the induction of acute mechano-hyperalgesia.
Supported by USPHS CA-080153.

Disclosures: A. Khodorova: None. **G. Strichartz:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Support: NIH NS078173

Title: The p75NTR signaling cascade mediates mechanical hyperalgesia induced by nerve growth factor injected into the rat hind paw

Authors: *G. D. NICOL[1], A. KHODOROVA[2], G. STRICHARTZ[2];
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Abstract: Recent studies show that NGF augments excitability of isolated rat sensory neurons through activation of the p75 neurotrophin receptor (p75NTR) and its downstream sphingomyelin signaling cascade, wherein neutral sphingomyelinase(s) (nSMase), ceramide, and the atypical PKC (aPKC), PKMζ, are key mediators. Here we determine if these same effectors participate in mechanical hypersensitivity caused by NGF. Mechanical sensitivity was quantitated by the number of paw withdrawals in response to 10 stimuli by a 4g von Frey hair (VFH, testing "allodynia") and by 10 g & 15 g VFHs (testing "hyperalgesia"). NGF (500 ng/10 μL) injected into the male rat's plantar hindpaw induced long-lasting ipsilateral mechanical hypersensitivity. The paw withdrawal frequency (n/10) for the strong VFHs increased significantly at 0.5-1.5 h after NGF injection, and remained elevated at 21-24h compared to both baseline responses and those of the contralateral paw. Injection of N-acetyl sphingosine (C2-ceramide, 20 μg/10 μL), a cell-permeant ceramide analog, induced ipsilateral "allodynia" that persisted for 24h, and an acute "hyperalgesia" that resolved by 2h after injection. Three consecutive injections of this dose of C2-ceramide, spaced 1.5 h apart, resulted in a significant hyperalgesia at 2 h, but not at 4 h, after the 3rd injection. Acute intraplantar pre-treatment with the nSMase inhibitors, glutathione and GW4869, prevented the acute hyperalgesia from NGF at 1.5h, but not that at 21-24h. Hyperalgesia was prevented by intraplantar injection of the myristolated pseudosubstrate inhibitor of PKCζ/PKMζ (mPSI, 40 μg/20 μL) 1d before NGF. Paw injection of a non-hydrolyzable form of the p75-selective agonist Pro-NGF gave virtually identical effects as NGF. The mechano-sensitizing effects of both Pro-NGF and NGF were prevented by pre-injection of the paw with the p75NTR blocking anti-body (gift of L. Reichardt, UCSF). These findings indicate that p75NTR and its activation of the sphingomyelin signaling cascade are essential for mechanical hypersensitivity resulting from NGF.

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Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Support: ANZCA AEG13/001

Title: Role of PCAF in chronic postsurgical pain

Authors: *Z. MENG, X. LIU, Y. TIAN, T. GIN, M. T. V. CHAN;
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Abstract: Objective:
There is emerging evidence to suggest that epigenetic modifier proteins (such as HDAC, P300) regulate pain perception. PCAF (P300/CBP Associated Protein), which is an epigenetic modifier, has been reported to interact with NF-κB and to stimulate COX-2 transcriptional activation. The aim of our study is to find out the direct impact of PCAF on pain modulation.
Method:
In our laboratory pain model, 0.1ml of CFA was injected in hindpaws of 8 SD rats. From Day 0 to Day 5, PCAF inhibitor anacardic acid (40 nmol/10ul) was injected intrathecally in 4 rats twice daily, while other 4 rats received saline. Behavioral tests, including Von Frey test and Hargreaves test, were performed 1h after anacardic acid injection.
The function of PCAF was also investigated in a clinical pain study of 1,152 post-surgical patients. Patients were genotyped for 49 SNPs in *PCAF* gene by GoldenGate genotyping assay. Haplotype combination was calculated by using Haploview 4.2. Patients were followed up at three months and one year after surgery and they were required to rate their pain score on a 0-10 scale.
Result:
In our rat CFA pain model, we found a trend that PCAF inhibitor anacardic acid can reduce both mechanical allodynia and thermal nociception. From Day 2 to Day 5, a higher mechanical threshold was observed in rats with anacardic acid treatment. Similarly, rats with anacardic acid showed longer nociceptive heat latency from Day 2 to Day 4. (Figure 1.)
In our pain database, patients with PCAF mutations reported altered chronic pain. Heterozygotes of rs6808352 experienced less pain at three months [0.59 (1.38) vs. 0.93 (1.84), P = 0.038]. Carriers of the minor allele at rs2948087 were more likely to have severe chronic pain (pain score > 5) at one year (OR = 11.62, 95% CI 2.99 -45.19, P < 0.001). Carriers with SNP at rs2948083 and a haplotype GAGG showed similar severe pain experience (P = 0.002 and < 0.001, respectively)
Conclusion:
Mutations in PCAF result in changed perception of post-surgical chronic pain in patients and inhibition of PCAF lead to reduction in inflammatory pain in rats.

Disclosures: Z. Meng: None. **X. Liu:** None. **Y. Tian:** None. **T. Gin:** None. **M.T.V. Chan:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Support: NIH grant - 1R01GM102575-01A1

Title: Further evidence that activation of AMPK inhibits acute and chronic post-surgical pain

Authors: *D. V. TILLU, M. N. ASIEDU, O. K. MELEMEDJIAN, T. M. HUGHES, G. DUSSOR, T. J. PRICE;
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Abstract: Surgery is a major cause of persistent pain suggesting that treatments that directly target the molecular pathology promoting post-surgical pain, particularly those that contribute to the progression to chronic pain, are needed. We have previously demonstrated that dysregulated protein translation regulation pathways, in particular ERK/eIF4E and mTOR signaling pathways underlie persistent pain states and that AMPK activators can profoundly inhibit ERK and mTOR signaling in sensory neurons. We have also demonstrated that local injection of resveratrol, a potent AMPK activator, into the hindpaw following plantar incision dose-relatedly reverses incision-mediated mechanical hypersensitivity as well as hyperalgesic priming induced by incision. The aim of the present study was to pharmacologically establish AMPK activation as a bona-fide mechanism for the alleviation of post-surgical pain. To do this, we utilized multiple AMPK activators, including metformin, OSU-53 and A-769662, that possess different mechanisms of AMPK activation to demonstrate a shared endpoint - inhibition of incision-induced mechanical hypersensitivity and hyperalgesic priming. Metformin, which is clinically available and widely prescribed, stimulates upstream LKB1 activity to activate AMPK whereas OSU-53 and A-769662 are positive allosteric modulators that directly activate AMPK. Using the Brennan incision model in mice, we demonstrate that systemic metformin or local OSU-53 injection dose-dependently and efficaciously attenuates incision-induced mechanical hypersensitivity as well as the development of hyperalgesic priming precipitated by hindpaw injection of PGE2 following resolution of incision-induced mechanical hypersensitivity. Interestingly, systemic A-769662 was not effective in blocking incision-induced acute mechanical hypersensitivity; however it significantly blocked hyperalgesic priming. This effect

was paralleled by lower doses of metformin, which had no acute effect yet blocked hyperalgesic priming. Finally, co-treatment with systemic metformin and local resveratrol at individually sub-efficacious doses at the time of incision blocked acute hypersensitivity and hyperalgesic priming suggesting potential super-additive effects of combined AMPK activator use. None of these treatment approaches adversely affected wound healing. These results provide further evidence for activation of AMPK as a novel treatment avenue for acute and chronic pain states induced by surgery. These preclinical findings afford the opportunity for immediate clinical testing due to the clinical availability of metformin.

Disclosures: D.V. Tillu: None. **M.N. Asiedu:** None. **O.K. Melemedjian:** None. **T.M. Hughes:** None. **G. Dussor:** None. **T.J. Price:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Topic: D.08. Pain

Support: NIH Grant NS065926

Title: Critical role of local translation and retrograde axonal transport of nascently synthesized CREB in IL-6-induced nociceptive sensitization

Authors: *O. K. MELEMEDJIAN[1], M. N. ASIEDU[2], J. K. MOY[2], T. J. PRICE[2];
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Abstract: Transcriptional regulation of genes by cAMP response element binding protein (CREB) is essential for the maintenance of long-term memory. Moreover, retrograde axonal trafficking of CREB in response to nerve growth factor is critical for the survival of developing primary sensory neurons. We have previously demonstrated that injection of the pro-inflammatory cytokine, interleukin-6 (IL-6), induces prolonged nociceptive sensitization which is prevented by the protein synthesis inhibitors. Moreover, treatment of primary sensory neurons in culture with IL-6 results in increased CREB levels. We hypothesized that retrograde axonal trafficking of nascently synthesized CREB might link local, activity-dependent translation to long-lasting changes in nociceptive sensitivity. To test this, we first determined if IL-6 enhances the expression of CREB and if it subsequently undergoes retrograde axonal transport, IL-6 injection into the mouse hindpaw caused an increase in CREB protein within 30 min and an increase in the sciatic nerve only after 2 hrs, consistent with retrograde transport. Importantly, co-injection of IL-6 with the methionine analogue azido-homoalanine (AHA), to assess nascently

synthesized proteins, demonstrated an increase in CREB containing AHA in the sciatic nerve 2 hrs post injection, indicating retrograde transport of nascently synthesized CREB. Behaviorally, knockdown of intraplantar CREB mRNA with siRNA, blockade of retrograde transport by disruption of microtubules or inhibition of dynein or intrathecal injection of cAMP response element (CRE) consensus sequence DNA oligos that act as decoys for CREB prevented the development of IL-6-induced mechanical hypersensitivity. Consistent with previous studies in inflammatory models, intraplantar IL-6 enhanced the expression of BDNF in dorsal root ganglion (DRG). This effect was blocked by inhibition of retrograde axonal transport and by intrathecal CRE oligos. Hence, these findings elucidate a novel mechanism of axonal translation and retrograde trafficking linking locally-generated signals to long-term nociceptive sensitization.

Disclosures: O.K. Melemedjian: None. **M.N. Asiedu:** None. **J.K. Moy:** None. **T.J. Price:** None.

Poster

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Topic: D.08. Pain

Support: NIH Grant NS073664

Title: Highly potent agonists reveal Protease Activated Receptor Type 2 (PAR2) -dependent hyperalgesic priming relying on central trkB/aPKC maintenance mechanisms

Authors: *T. J. PRICE[1], D. V. TILLU[2], M. N. ASIEDU[2], S. BOITANO[3], J. VAGNER[4]; [1]Pharmacol., Univ. Arizona, TUCSON, AZ; [2]Pharmacol., [3]Physiol., [4]Bio5 Inst., Univ. of Arizona, Tucson, AZ

Abstract: Protease Activated Receptor Type 2 (PAR2) is a G-protein coupled receptor (GPCR) containing a tethered ligand in the N-terminal domain that is exposed upon protease digestion of the N-terminal domain. This peptide sequence, SLIGRL in rodents, has served as a basis for peptide ligand discovery at the native receptor capable of bypassing proteolytic cleavage of the N-terminal domain. We have developed a wide range of highly potent and efficacious agonists to probe PAR2 function in vitro and in vivo. PAR2 is thought to play an important role in inflammatory- and cancer-evoked pain based on studies using PAR2-/- mice. Recently hyperalgesic priming has emerged as important model system for probing plasticity in the nociceptive system. We have shown that the maintenance of hyperalgesic priming evoked by a

single injection of interleukin-6 relies on a dorsal horn signaling axis involving Brain Derived Neurotrophic Factor (BDNF) signaling via trkB to atypical PKC (aPKC). Here we have tested the hypothesis that specific activation of PAR2 should be capable of evoking hyperalgesic priming. We have further tested whether the maintenance of this priming involves a BDNF/trkB/aPKC signaling axis. We find that intraplantar injection of the potent and specific PAR2 agonist, 2-aminothiazol-4-yl-LIGRL-NH2 (2at-LIGRL), evokes a long-lasting acute allodynia (EC50 ~ 0.03 nmoles) that is followed by a profound hyperalgesic priming to subsequent prostaglandin E2 (PGE2) injection. The pro-allodynic effect of 2at-LIGRL is completely absent in PAR2-/- mice as is hyperalgesic priming. Hence, stimulation of PAR2 is sufficient to evoke hyperalgesic priming in mice. We then asked if the maintenance of this hyperalgesic priming can be reversed by inhibition of BDNF/trkB/aPKC signaling. Systemic dosing with the trkB antagonist ANA-12 (0.5 mg/kg) following the resolution of acute 2at-LIGRL-induced allodynia inhibited priming precipitated by PGE2 injection into the hindpaw. Likewise, injection of the aPKC inhibition, ZIP, into the lumbar spinal cord completely reversed the maintenance of priming over the same time course. Hence, PAR2 activation is sufficient to evoke hyperalgesic priming. Moreover, the maintenance of this primed state is dependent on a CNS BDNF/trkB/aPKC signaling axis suggesting a generalized role for this signaling pathway in maintenance of hyperalgesic priming.

Disclosures: T.J. Price: None. **D.V. Tillu:** None. **M.N. Asiedu:** None. **S. Boitano:** None. **J. Vagner:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Program#/Poster#: 266.24/UU10

Topic: D.08. Pain

Title: Nociceptive sensitization by activated Proreinase-activated receptor 2

Authors: *K. KIDO, E. MASAKI;
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Abstract: Background- Peripheral mast cells degranulate and its number are largely reduced following tissue injury. Furthermore, we previously indicated that the prevention of mast cell degranulation reduced guarding pain and mechanosensitivity in a postoperative pain model of mice. Proteinase-activated receptor 2 (PAR-2) is a G protein-coupled receptor that is expressed in the peripheral sensory neurons and may play an important role in inflammatory and injured

pain by binding with tryptase from mast cells.In this study, we investigated effects of PAR-2 agonist SLIGRL-NH2 on the plantar skin of the rat hindpaw.
Method- Using the rat glabrous in vitro skin-tibial nerve preparation, afferent nerve activities from single mechanosensitive nociceptors were recorded. Spontaneous activities, and responses to mechanical stimuli (5, 10, 20, 40, 80 and 120 mN) were recorded before and after SLIGRL-NH2 application or phosphate buffered saline (PBS) control. Responses to heat and cold stimuli (From 12°C to 47°C) were recorded once after exposure to either with SLIGRL-NH2 application or PBS control.
Results- 100μM SLIGRL-NH2 increased spontaneous activities in 8 of 18 C-fibers (44.4%) within 5 minutes. SLIGRL-NH2 application did not change the responses to mechanical stimuli in C- fibers. C-fibers were sensitized to heat and cold stimuli: 1) The percentage of fiber that responded to heat (70.8% vs. 53.3%) and cold (84.6% vs. 54.5%) was significantly increased respectively; 2) Cold thresholds were significantly decreased (21.0°C vs. 27.0°C); 3) Heat thresholds and total action potentials during the heat stimulus were not significantly differences from control.
Conclusion- PAR-2-mediated excitation and sensitization of peripheral primary nociceptors may contribute to PAR-2-mediated pain, such as postoperative pain.

Disclosures: K. Kido: None. **E. Masaki:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

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Title: Opioid-induced hyperalgesia contains a peripheral component

Authors: *M. P. ROWAN[1], S. M. BIERBOWER[2], M. A. ESKANDER[3], E. D. POR[1], R. GOMEZ[1], N. A. JESKE[1];
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Abstract: Chronic pain is a critical national health problem and the most common cause of long-term disability, affecting over a third of the population. Drugs acting at mu opioid receptors

(MOR) are essential in the treatment of moderate to severe chronic pain. However, prolonged use of opioids can lead to severe side effects including tolerance and dependence that are largely absent in animals lacking β-arrestin2. Chronic treatment with opioids can also lead to opioid-induced hyperalgesia (OIH), characterized by exaggerated pain sensitivity and diffusivity. OIH is difficult to treat, in large part because the cellular mechanisms underlying OIH are poorly understood. Recent evidence has shown a crucial role for the transient receptor potential vanilloid type 1 (TRPV1) channel in OIH. TRPV1 is a sensor of multiple forms of noxious stimuli, is co-expressed on primary sensory neurons with MOR, and displays enhanced responsiveness following chronic morphine treatment. We have recently shown that TRPV1 is desensitized by β-arrestin2, so we hypothesize that chronic opioid treatment leads to TRPV1 sensitization following β-arrestin2 recruitment to MOR in primary sensory neurons.
Primary sensory neurons from rat trigeminal ganglia (TG) were nucleofected with MOR-GFP and assessed for TRPV1 activation following capsaicin-mediated stimulation. Pretreatment with DAMGO or morphine, MOR agonists that recruit β-arrestin2 in TGs as verified by TIRF-FRET, significantly enhanced the TRPV1 response. Pretreatment with herkinorin, a MOR agonist that does not recruit β-arrestin2, had no effect on the TRPV1 response. Additionally, the DAMGO-mediated increase in TRPV1 response was absent following siRNA knockdown of β-arrestin2. Treatment of TG neurons with morphine or DAMGO, not herkinorin, reduced the interaction of TRPV1 and β-arrestin2. Rats and mice injected chronically with peripherally restricted doses of morphine and DAMGO, not herkinorin, developed OIH. TRPV1 knockout mice did not develop OIH. Together these data suggest that β-arrestin2 recruitment to MOR, away from TRPV1, may play a peripheral role in the development and/or maintenance of OIH.

Disclosures: M.P. Rowan: None. **S.M. Bierbower:** None. **M.A. Eskander:** None. **E.D. Por:** None. **R. Gomez:** None. **N.A. Jeske:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 266.26/UU12

Topic: D.08. Pain

Support: Medical Research Service, Dept. Veterans Affairs

Rehabilitation Research Service, Dept. Veterans Affairs

Paralyzed Veterans of America

Title: Virus-mediated knockdown of Nav1.3 in dorsal root ganglia of diabetic rats alleviates mechanical pain

Authors: *O. A. SAMAD, A. M. TAN, S. D. DIB-HAJJ, S. G. WAXMAN;
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Abstract: Pain affects a substantial number of people with diabetic neuropathy, making it a major public health problem. To date, available clinical treatments remain only partially effective against diabetic neuropathy, and are accompanied by important side effects. We have shown that voltage-gated sodium channel Nav1.3 is upregulated in Dorsal Root Ganglion (DRG) neurons of diabetic rats that develop pain. Moreover, this upregulation correlates over time with the decrease in mechanical thresholds of diabetic rats. Since Nav1.3 upregulation drives neuronal hyperexcitability, and its suppression in rats results in attenuation of neuropathic pain due to peripheral nerve injury, we predict that knockdown of Nav1.3 in painful diabetic neuropathy can attenuate pain. To test this hypothesis, we used a validated recombinant Adeno-Associated Virus AAV-shRNA-Nav1.3 viral vector (Samad et al., 2013) to knockdown Nav1.3 in DRG of diabetic rats. We asked the following specific question: Is knockdown of Nav1.3 within multiple DRG neurons in vivo via AAV-shRNA effective in attenuating pain due to diabetes?
To induce diabetic neuropathy in rats, we used the Streptozotocin (STZ)-induced diabetes model. We and others have shown that this established model of diabetes induce neuropathic pain symptoms in rats, and is therefore well-suited to test potential pain-reducing therapies. We used a clinically applicable intrathecal injection method of AAV-shRNA-Nav1.3 to knockdown Nav1.3 in multiple DRGs of diabetic rats and compared them to rats injected with control AAV-shRNA-NT (Non-Targeting shRNA control). The viral vectors were administered intrathecally on the fourth day after STZ-injection and the animals analyzed over a period of one month. Our preliminary results indicate that Nav1.3 knockdown in DRG attenuates mechanical allydonia in diabetic rats. Other ongoing outcome measures being assessed include: cFos expression analysis and excitability of wide dynamic range neurons in the dorsal horn, and assessment of efficacy and specificity of viral infection. Our study offers a promising approach to gene therapy for painful diabetic neuropathy.

Disclosures: O.A. Samad: None. **A.M. Tan:** None. **S.D. Dib-Hajj:** None. **S.G. Waxman:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 266.27/UU13

Topic: D.08. Pain

Support: NIH Grant DE018252

Title: The functional implications of the differential distribution of na+/ca2+ exchanger isoforms in rat sensory neurons

Authors: *N. SCHEFF, E. YILMAZ, M. S. GOLD;
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Abstract: We have shown previously that depolarization-evoked Ca2+ transients differ greatly among subpopulations of dorsal root ganglion neurons, in association with the differential contribution of Ca2+ regulatory mechanisms. One of these Ca2+ regulatory mechanisms, the Na+/Ca2+ exchanger (NCX), appears to play a selective role in the regulation of the duration of the high K+-evoked Ca2+ transient in IB4-binding and capsaicin sensitive small and medium diameter DRG neurons. The purpose of the present study was to 1) further define the subpopulation of neurons in which NCX functions, 2) identify the isoform(s) underlying NCX activity, and 3) begin to assess the function of this isoform in vivo. In retrogradely labeled neurons from the glabrous skin of adult male Sprague-Dawley rats, evidence of NCX activity, as assessed with non-specific block with replacement of Na+ with Li+ or choline, was restricted to a subpopulation of small diameter IB4+, largely capsaicin sensitive neurons. While single cell PCR analysis suggested that mRNA encoding all three NCX isoforms (NCX1-3) was detectable in this subpopulation of cutaneous neurons with NCX3 below the level of detection in ~40% of neurons assessed, Ca2+ imaging data with NCX1 and 3 selective blockers SN-6 and KB-R7943, respectively, suggested that NCX3 was responsible for >85% of NCX activity, and NCX2 was responsible for the remainder. Interestingly, western blot analysis suggested both the presence of sensory neuron-specific NCX3 splice variants and differential trafficking of NCX3 splice variants to central and peripheral terminals. We have evidence to suggest NCX is not only present in peripheral C-fiber axons but appears to primarily function in reverse mode, contributing to the maintenance of a hyperpolarized membrane potential, secondary to the influx of Ca2+. Finally, small interfering RNA (siRNA) was utilized to knock down each isoform in vivo. Knock-down of NCX3 resulted in a decrease in both mechanical and thermal nociceptive threshold, suggesting a role for NCX3 in the regulation of transmitter release from nociceptive afferents. Ongoing studies are being done to determine the functional consequences of the differential trafficking of NCX3 to the peripheral and central terminals.

Disclosures: N. Scheff: None. **E. Yilmaz:** None. **M.S. Gold:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 266.28/UU14

Topic: D.08. Pain

Support: NINDS R01 NS071952

Title: Neto protein assembly with kainate receptors in sensory neurons

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Abstract: Ionotropic glutamate receptors (iGluRs) are mediators of nociceptive signaling throughout the pain neuraxis. Two families of iGluRs, AMPA and NMDA receptors, contribute substantially to excitatory signaling and plasticity in pain pathways, but therapeutic targeting of these receptors for pain (or neurological disease) is known to have debilitating side effects that to date preclude their clinical use. A third family of iGluRs, the kainate receptors (KARs, formed from GluK1-GluK5 subunits), represent more promising therapeutic targets. KARs are expressed throughout the peripheral and central nervous system, including in those pathways comprising the pain neuraxis. Minimal side effects have been reported with effective doses of KAR antagonists in both healthy volunteers and migraine sufferers. These human studies support the findings of more than a decade of animal studies which show KARs, and in particular GluK1-containing receptors, to be involved in the mediation of allodynia and hyperalgesia following peripheral inflammation or nerve injury in rodents.

KARs are expressed in dorsal root ganglion (DRG) neurons, and in the spinal dorsal horn neurons that comprise the targets of DRG innervation. In DRG, KARs are expressed predominantly in IB4-positive C-fiber nociceptors and serve at least two functions: (i) peripheral chemosensing and (ii) presynaptic modulation of glutamate release from afferent terminals in the dorsal horn of the spinal cord. Recently, neuropilin and tolloid-like 1 (Neto1) and Neto2 were shown to profoundly modulate recombinant and endogenous KARs in receptor subunit- and Neto isoform-dependent fashions, and therefore could be important but poorly characterized constituents of KAR function in sensory pathways. We find that KAR kinetics are altered in Neto-null DRG neurons, suggesting functional assembly of Neto with native receptors in peripheral sensory neurons. Given this coassembly, we are examining the impact of Neto loss on KAR function in peripheral and central DRG projections. Despite the demonstrated importance of GluK1 in formalin-induced spontaneous pain and hypersensitivity, we find these pain modalities to be unaffected by genetic deletion of Neto proteins and are investigating the role of Neto in other pain behaviors.

Elucidation of how Neto proteins shape KAR function in sensory signaling pathways is key for

developing a clear mechanistic understanding of the relevance of KARs to sensory transmission and their potential utility as therapeutic targets.

Disclosures: C. Vernon: None. **G.T. Swanson:** None. **B.A. Copits:** None.

Poster

266. **Nociceptors: Molecular and Pharmacological Studies**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 266.29/UU15

Topic: B.04. Ion Channels

Support: NSFC 30930044

Title: Ligand-induced membrane delivery of $P2X_3$ receptor in primary sensory neurons

Authors: *L. BAO[1], X.-Q. CHEN[1], J.-X. ZHU[1], Y. WANG[1], X. ZHANG[2];
[1]Inst. of Biochem. and Cell Biology, Chinese Acad. of Sci., Shanghai, China; [2]Inst. of Neurosci. and State Key Lab. of Neuroscience, Inst. for Biol. Sciences, Chinese Acad. of Sci., Shanghai, China

Abstract: ATP-gated channel $P2X_3$ receptor is mainly expressed in primary sensory neurons and responsible for pain processing. Increased membrane insertion and functional upregulation confers $P2X_3$ receptor sensitization under pathological conditions. Multiple signaling pathways are involved in the regulation of $P2X_3$ receptor function by various inflammation mediators under pathological conditions. Our previous study has reported that ATP potentiates endocytosis and retrograde transport of $P2X_3$ receptor in the axons of primary sensory neurons. Here, we also showed that ATP promoted membrane delivery of $P2X_3$ receptor in a time-dependent manner. $P2X_3$ receptor-mediated Ca^{2+} influx-activated Ca^{2+}/calmodulin-dependent protein kinase II (CaMKIIα) regulated the membrane insertion of $P2X_3$ receptor in a tertiary structure-dependent manner. The N terminal of $P2X_3$ receptor was responsible for binding with CaMKIIα, whereas Thr388 in the C terminal was phosphorylated by CaMKIIα. Thr388 phosphorylation increased the interaction between $P2X_3$ receptor and caveolin-1, leading to potentiated membrane insertion of $P2X_3$ receptor. Moreover, the regulated forward trafficking of $P2X_3$ receptor by ligand also drove membrane delivery of $P2X_2$ receptor which co-assembles with $P2X_3$ receptor. Together, we present a novel mechanism responsible for the ligand-induced membrane delivery of $P2X_3$ receptor.

Disclosures: L. Bao: None. **X. Chen:** None. **J. Zhu:** None. **Y. Wang:** None. **X. Zhang:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.01/UU16

Topic: D.08. Pain

Support: JSPS Grant-in-Aid for Young Scientists (B) Grant #24791624

Title: Neurotropin suppresses lidocaine-induced neurotoxicity in cultured dorsal root ganglion neurons

Authors: *R. ISONAKA[1], T. TAKENAMI[2], T. KATAKURA[1], T. KAWAKAMI[1];
[1]Physiol., [2]Anesthesiol., Kitasato Univ. Sch. of Med., Sagamihara, Japan

Abstract: Neurotropin is an analgesic agent, and the main ingredient of this drug is a non-protein extract from inflamed skin of rabbits inoculated with vaccinia virus. It is used for the treatment of various chronic pains, and some reports showed that neurotropin affect the axonal degeneration induced by the drug for colorectal cancer. Lidocaine is a major local anesthetics, it is used for the treatment of allodynia and chronic pains in the field of orthopedics, neurology and anesthesia. However, lidocaine is known to have neurotoxicity and inhibit axonal transport. We hypothesized that neurotropin relieves lidocaine-induced inhibition of axonal transport. To test our hypothesis, we investigated that the effect of a combination with lidocaine and neurotropin on axonal transport in cultured mouse dorsal root ganglion neurons. We used the video-enhanced microscopy, which allowed us to observe the real-time dynamics of axonal transport. Simultaneous addition of neurotropin significantly blocked the lidocaine-induced inhibition of axonal transport. These results suggested that neurotropin may lead to suppress lidocaine-induced neurotoxicity.

Disclosures: R. Isonaka: None. **T. Takenami:** None. **T. Katakura:** None. **T. Kawakami:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.02/UU17

Topic: D.08. Pain

Support: NIH Grant R01-DA033059

VA Rehabilitation Grant 1I01RX000378

Title: NMDA receptors in primary afferents are potentiated by BDNF released by microglia during the induction of neuropathic pain

Authors: *J. G. MARVIZON[1], W. CHEN[1], H. S. ENNES[1], W. WALWYN[2], J. A. MCROBERTS[1];
[1]Dept. of Med., [2]Dept. of Psychiatry and Biobehavioral Sci., UCLA, LOS ANGELES, CA

Abstract: We previously found that BDNF induces the activating phosphorylation of the NR2B subunit of NMDA receptors in primary afferents, increasing NMDA-induced neurokinin 1 receptor (NK1R) internalization, a measure of substance P release. Since spinal cord microglia release BDNF during the onset of neuropathic pain, we hypothesized that these NMDA receptors become potentiated after nerve injury. To confirm this, we gave rats chronic constriction injury (CCI) of the sciatic nerve and intrathecal NMDA (10 nmol, with 10 nmol D-Ser) at various times thereafter. Measures of hind paw withdrawal to von Frey hairs confirmed that allodynia developed during the first two days after CCI. Ipsilaterally to CCI, there was a marked increase in NMDA-induced NK1R internalization that peaked 6 hr after CCI and lasted 3 days. Contralaterally, NK1R internalization increased in days 2 and 3 to values similar to the ipsilateral side. Intrathecal saline after CCI or intrathecal NMDA after sham surgery resulted in negligible NK1R internalization, showing that substance P release required both NMDA receptor activation and nerve injury. To investigate the signals involved in NMDA receptor potentiation, rats were given microglia inhibitors (200 nmol minocycline, 1 nmol fluorocitrate or 10 µg propentofylline), the BDNF scavenger trkB-Fc (10 µg), the trkB antagonist ANA-12 (100 nmol), the Src family kinase inhibitor PP2 (10 nmol) or saline. These compounds were injected intrathecally twice: immediately after CCI and 3 hr later; followed by intrathecal NMDA 6 hr after CCI. All of the compounds inhibited NMDA-induced NK1R internalization. We also determined whether activation of microglia with lipopolysaccharide (LPS) increases NMDA-induced NK1R internalization. We gave rats intrathecal LPS (2 µg) or saline twice, 24 hr apart; and NMDA or saline 6 hr after the second injection. LPS induced microglia activation, measured as staining for the microglia marker Iba-1 in the central dorsal horn. NK1R internalization was high when NMDA was given after LPS, but not when NMDA was given after saline, when LPS was followed by saline, or in the saline-saline controls. NMDA-induced NK1R internalization peaked 6 hr after LPS and disappeared by 24 hr. The increase in NMDA-induced NK1R internalization produced by LPS was decreased by trkB receptor antagonist ANA-12, the BDNF scavenger trkB-Fc or the Src family kinase inhibitor PP2. Therefore, NMDA receptors in primary afferent terminals are potentiated during the induction of neuropathic pain as the result

of BDNF release from microglia, activation of trkB receptors and phosphorylation of the NR2B subunit by a Src family kinase.

Disclosures: J.G. Marvizon: None. **W. Chen:** None. **H.S. Ennes:** None. **W. Walwyn:** None. **J.A. McRoberts:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.03/UU18

Topic: D.08. Pain

Support: NIH Grant R01-DA033059

Title: BDNF increases NMDA receptor currents in primary afferent neurons by inducing phosphorylation of Tyr1472 of the NR2B subunit

Authors: *J. A. MCROBERTS[1], W. WALWYN[2], H. S. ENNES[1], W. CHEN[1], J. MARVIZON[1];
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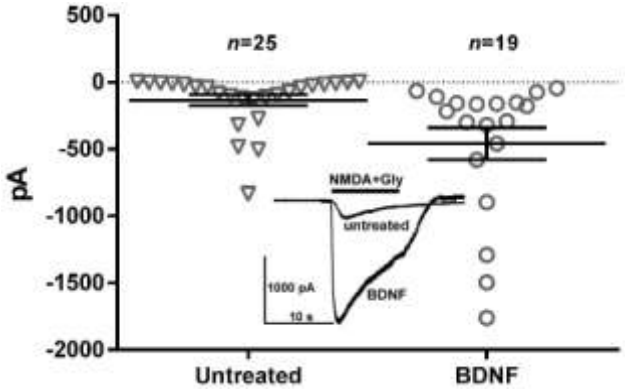
Abstract: NMDA receptors are present in the central and peripheral terminals of primary afferent neurons. Previously, we found that the ability of NMDA to induce substance P release from the central terminals of primary afferents was markedly increased by BDNF. Using dorsal root ganglion (DRG) neurons from adult rats cultured for 2-3 days, we investigated whether BDNF increased NMDA receptor currents, and induced the phosphorylation in Tyr1472 of the NR2B subunit, which is known to increase NMDA receptor activity. Prior to whole cell patch-clamp recording, DRG neurons were left untreated or were pre-treated with BDNF (30 ng/ml) for 1-2 hr. Once the cells had stabilized at a holding potential of -70 mV, NMDA (250 μM) and glycine (10 μM) were applied for 10 s by pressure ejection and the peak current measured. BDNF increased the NMDA/Gly induced inward current from -149 ± 45 pA to -459 ± 119 pA ($p = 0.0168$, t-test, Figure). There was also an increase in the number of cells that responded to NMDA/Gly above 50 pA: untreated, 50 %, n = 25; BDNF, 95 %, n = 19 ($p = 0.001$, Fisher's exact test). There was no difference in the size of the cells recorded as assessed from cell capacitance (untreated: 41 ± 4 pF, BDNF: 49 ± 5 pF). To determine if BDNF increases tyrosine phosphorylation of the NR2B subunit, cultured DRG neurons were left untreated or treated with 20 ng/ml BDNF for 1 hr, then harvested and protein extracts prepared for electrophoresis. Western blots were first probed with an antibody to phospho-Tyr1472-NR2B, then stripped and reprobed with antibodies to total NR2B and ß-actin. Although there was NR2B phosphorylation

in control cultures, BDNF caused a 2.1 ± 0.4-fold increase in phospho-NR2B ($p < 0.008$; t-test). Therefore, BDNF increases NMDA receptor currents in primary afferents by inducing the phosphorylation of Tyr1472 of the NR2B subunit. In related experiments that measured NMDA-induced substance P release, we found that this phosphorylation is mediated by activation of trkB receptors and a Src family kinase. The potentiation of NMDA receptors in primary afferents by BDNF may contribute to neuropathic/chronic pain.



Disclosures: J.A. McRoberts: None. **W. Walwyn:** None. **H.S. Ennes:** None. **W. Chen:** None. **J. Marvizon:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.04/UU19

Topic: D.08. Pain

Support: NIH Grant AR056288

Title: A role for spinal brain-derived neurotrophic factor in persistent pain in a rat model of mechanical facet joint injury

Authors: *J. KRAS, C. WEISSHAAR, J. QUINDLEN, B. WINKELSTEIN;
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Abstract: Neck and back pain represent the most frequent causes of job-related disability, with costs exceeding $50 billion annually. The facet joint is a common source of neck and low back pain and is susceptible to mechanical injury. Although animal studies support tensile loading of the cervical facet capsular ligament as a cause of persistent pain and report associated upregulation of pain-related molecules, such as substance P, and induction of neuronal

hyperexcitability, the mechanism(s) of pain from this joint injury are still undefined. Changes in brain-derived neurotrophic factor (BDNF) levels are implicated in a host of painful conditions, but the relationship between BDNF and injury-induced joint pain also remains unknown. In this study, we defined the BDNF response in both the dorsal root ganglia (DRG) and spinal cord following painful cervical facet joint loading; we also evaluated if there is a functional contribution of BDNF in the spinal cord to the maintenance of persistent pain that develops from joint injury. Separate groups of male Holtzman rats underwent a painful bilateral C6/C7 facet joint distraction injury or sham surgery. Bilateral forepaw mechanical hypersensitivity was assessed using von Frey filaments applied to the plantar surface of the forepaws. In separate rats after behavioral testing on day 1 or day 7, the C6/C7 spinal cord and DRG were harvested, and BDNF mRNA and protein levels were quantified using real-time RT-PCR and immunohistochemistry, respectively. Facet joint distraction induced significant ($p<0.001$) mechanical hypersensitivity at both time points. BDNF mRNA was unchanged in the DRG after painful injury compared to sham levels at both time points, but BDNF protein significantly increased ($p<0.016$) over sham in the DRG at day 7. Both BDNF mRNA ($p=0.031$) and protein ($p=0.047$) significantly increased in the spinal cord at day 7 after painful injury. In a separate study, sequestering endogenous BDNF via intrathecal administration of trkB-Fc on day 5 after injury returned the forepaw withdrawal threshold to baseline levels on days 6 and 7. Yet, administration of the matched vehicle IgG-Fc did not change the withdrawal threshold after injury. Additionally, the expression of pERK1/2 relative to total ERK1/2 in the spinal cord at day 7 was significantly reduced ($p<0.045$) in the trkB-Fc treated group compared to the vehicle group. In the context of increased spinal BDNF after painful mechanical facet joint injury, this reduction in ERK activation coupled with the reduction in pain that is achieved by spinal BDNF sequestration suggests ERK-mediated contributions of spinal BDNF in the maintenance of mechanical joint pain.

Disclosures: J. Kras: None. **C. Weisshaar:** None. **J. Quindlen:** None. **B. Winkelstein:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

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Topic: D.08. Pain

Support: DOD grant #W81XWB-10-2-0140

Title: Painful whole body vibration induces increased expression of nerve growth factor & brain-derived neurotrophic factor in cervical intervertebral discs in a rat model

Authors: M. E. ZEEMAN, S. KARTHA, H. A. BAIG, B. B. GUARINO, *B. A. WINKELSTEIN;
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Abstract: Chronic neck pain is a common disorder with high costs, affecting many in the general population. Discogenic pain is a common source of pain and is hypothesized to originate when the typically aneural intervertebral disc (IVD) becomes innervated after injury or with degeneration. Spinal injury from whole body vibration (WBV) has been linked to low back and neck pain, with increased loading in the vertebral column and discs speculated as a potential pain source. Yet, the biochemical mechanisms leading to discogenic pain from its hyper-innervation and/or degeneration are unclear. Studies report upregulation of the neurotrophins, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), in lumbar degenerated IVDs. Because these growth factors mediate nerve growth they are believed to contribute to disc pain. Although there is increasing focus on defining the role of these factors in discogenic pain, work has largely focused on the lumbar discs, and no study has determined if similar mechanisms exist in the cervical spine. This study aimed to define if painful WBV induces changes in neurotrophins in the cervical IVDs. WBV was imposed along the spine's long-axis under inhalation anesthesia with the rat in the prone position. Vibration was applied for 30 minutes at 15Hz and 0.55g daily for 7 days followed by 7 days of rest. Sham rats underwent the same paradigm with anesthesia exposure only. Mechanical hyperalgesia was assessed prior to, during, and after the WBV exposure period. On day 14, cervical spines were harvested to evaluate NGF and BDNF mRNA and protein expression using RT-qPCR and western blot (n=8/group). Immunohistochemistry (n=4/group) was used to localize and quantify NGF and BDNF expression levels in different regions of the disc. WBV exposure induced behavioral hypersensitivity in the forepaw through day 14 that was not present in shams ($p<0.01$). In association with pain, WBV increased NGF and BDNF transcripts by 1.6-fold and 2.0-fold over sham, but not significantly. WBV did produce a significant ($p<0.006$) 5-fold increase in BDNF protein and a significant ($p<0.04$) 1.8-fold increase in the 75kDa NGF isoform. The 28kDa isoform of NGF increased 4.9-fold, but this was not significant. After WBV, both NGF and BDNF labeling significantly ($p<0.01$) increased in the inner annulus compared to the outer annulus of the disc. These findings suggest that painful WBV may induce similar patterns of upregulation of neurotrophins as in disc degeneration. Results also suggest that the same painful processes present in the lumbar spine may occur in the cervical spine and may explain one such potential mechanism responsible for the development of chronic neck pain.

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Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.06/UU21

Topic: D.08. Pain

Support: KAKENHI 22689043

Title: Methylation of BDNF genes in rat oro-facial neuropathic pain model~possible biomarker of neuropathic pain in the future~

Authors: *A. NAKAE, K. NAKAI, T. TANAKA, Y. ISHIDA, S. ISHINO, K. HOSOKAWA, T. MASHIMO, Y. FUJINO;
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Abstract: Background and Goal of the study:
Pain is a subjective symptom and the mechanisms are unclear. Thus, chronic pain, especially neuropathic pain, is diagnosed by patient symptoms rather than an objective test. One of the most important requirements is an objective useful biomarker.
Brain-derived neurotrophic factor (BDNF) can potentiate pain transduction from primary sensory neurons to spinal dorsal horn in response to nerve injury and is well known that DNA methylation affects transcription of BDNF.
Towards that end, we examined the methylation profile of the BDNF in trigeminal ganglion after infra-orbital nerve (ION) injury as an orofacial neuropathic pain model to identify an appropriate epigenetic biomarker for the objective diagnosis of neuropathic pain.
Material and Methods:
We used ION loose ligation model using SD rats (N=8 each). 28 day after surgery when injured animal expressed pain behavior, the trigeminal ganglion samples were taken from each animal. All methylation analysis was performed using Solid4 system (Life Technologies, CA). Clustering analyses were performed using JMP 9.0.1 software, with the Ward's method. The difference in the methylation rates was compared by one-way ANOVA and statistical differences were resolved post-hoc using Tukey-Kramer multiple-comparison test ($p<.05$).
Results and Discussion:
28days after injury, pain threshold values from injured rats were statistically significantly lower than from sham and naïve rats.
The methylation rates are shown in table and analyses of the dendrogram from methylation profiles of exon I, II, VI, and VII each promoter region demonstrated that classification of nerve injured rats and naïve controls at the first branch completely matched the condition. Our results

indicate that classification based on the DNA methylation profiles of BDNF gene may be a valuable diagnostic biomarker for neuropathic pain.
Conclusion:
Based on analyses of the methylation profiles within the CpG island at the promoter of the BDNF gene in trigeminal ganglion, we could accurately classify animals into two groups (nerve-injured and naïve).
Ackowledgement
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Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

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Program#/Poster#: 267.07/UU22

Topic: D.08. Pain

Support: GACR305/09/1228

P304/12/G069

LH12058

CZ.1.07/2.3.00/30.0025

RVO67985823

Title: The role of AT1 R in the development of pain hypersensitivity after peripheral nerve injury

Authors: N. KALYNOVSKA, M. DIALLO, *J. PALECEK;
Dept. of Functional Morphology, Inst. of Physiol., Prague, Czech Republic

Abstract: Neuropathic states are often characterized by chronic pain and increased sensitivity to innocuous and noxious mechanical and thermal stimuli, allodynia and hyperalgesia. Neuroinflammatory changes at the spinal cord level are considered to be one of the possible mechanisms leading to the neuropathic pain states after the peripheral nerve injury. Several

studies have documented that Angiotensin II, the main effector molecule of Renin-Angiotensin system (RAS), is involved in neurodegenerative processes due to its proinflammatory effects. In this study the role of Angiotensin II receptor type 1 (AT1 R) signaling in the development and maintenance of neuropathic pain was investigated using AT1 R antagonist losartan. Male adult Wistar rats were used with the L5 spinal nerve ligation (SNL) model of peripheral neuropathy. Electronic von Frey mechanical stimulation and heat plantar test were used to determine withdrawal threshold levels for mechanical and thermal stimulation. Behavioral tests were performed before SNL to register the control value and then 1, 3, 5, 7, 9, 12 and 14 days after the SNL operation. Neuropathic rats were treated either with systemic (100 mg/kg) or intrathecal (20µM/10µl) administration of losartan. SNL caused significant reduction of the threshold to the heat stimulus reflected by reduced paw withdrawal latency (PWL). Maximal decrease of PWL on the side ipsilateral to the SNL was detected on the 9th postoperative day (16.5±0,1s) when compared to the control value (22, 8±0,1s, ($P \leq 0,001$)) and to the contralateral side (20,3±0,2 s, ($P \leq 0,001$)). Reduced PWL persisted till the end of the experiment and indicated thermal hyperalgesia. Treatment with losartan significantly attenuated changes in PWL on the 9th postoperative day (20,3±0,2 s ($P \leq 0,001$)). However, losartan treatment did not affect mechanical allodynia, induced by the SNL. No significant difference between the systemic and intrathecal losartan administration was observed. These results suggest that blockade of the AT1 R attenuates thermal hyperalgesia in the neuropathic pain model, presumably at the spinal cord level. Further experiments are needed to confirm therapeutic effect of AT1 R antagonists and to investigate the role of AT1 R signaling in initiation and maintenance of pain hypersensitivity after nerve injury.

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Disclosures: N. Kalynovska: None. **J. Palecek:** None. **M. Diallo:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.08/UU23

Topic: D.08. Pain

Support: GACR305/09/1228

P304/12/G069

LH12058

CZ.1.07/2.3.00/30.0025

RVO67985823

Title: Application of Angiotensin II-Receptor 1 antagonist (Losartan) attenuates spinal cord neuroinflammation in a model of neuropathic pain

Authors: *M. S. DIALLO, N. KALYNOVSKA, J. PALECEK;
Acad. of Sci. of Czech Republic, Praha, Czech Republic

Abstract: It is now well documented that the immune system and neuroinflammatory changes at the spinal cord level are widely involved in the initiation and maintenance of neuropathic pain present after nerve injury. Recently, it has been shown that the angiotensin II-receptor 1 (AT1R), receptor of the major Renin-Angiotensin System (RAS) effector, may play a role in neuroinflammatory processes in the CNS. In our study, the effect of losartan (AT1R antagonist) application was tested in a model of neuropathic pain in rats induced by spinal nerve ligation (SNL). Behavioral tests were used to confirm the presence of increased sensitivity to mechanical and thermal stimuli after the SNL. Western blot analysis was used to quantify and compare the expression levels of neuroinflammatory markers in the spinal cord L4-L6 lumbar segments, in the SNL rats on the operated (ipsilateral) and the controlateral sides at the 7th post-operative day. Our data showed that SNL evoked an upregulation of different proinflammatory factors and their receptors on the ipsilateral side when compared to the contralateral half of the spinal cord segment. Chemokine C-C motif ligand 2 (CCL2) and its receptor CCR2; and the cytokine tumor necrosis factor-α (TNF-α) and its receptor TNFR1 were increased to 144.8 ± 22.9 %, 156.7 ± 10.1, 128.3 ± 3.7 and 139.2 ± 8.3 %; respectively. SNL injury also induced, as expected, a microglial activation documented by strong increase of microglial marker OX42 levels in the ipsilateral spinal cord to 174.9 ± 31.4%. The levels of the AT1R protein expression were also increased on the ipsilateral side 158.1 ± 15.4 %. In another group of animals chronic treatment with losartan per os (100 mg/kg) was used during and after the SNL model induction. The expression level of the inflammatory factors in this group was not different when the ipsilateral and contralateral sides were compared: CCL2: 93.9 ± 4.4 %; CCR2: 102.2 ± 7.7 %; TNF-α: 83.8 ± 8.9 %; TNFR1: 88.2 ± 3.3 %; OX42: 108.3 ± 6.1 %; AT1R: 93.9 ± 10.5. Our results suggest that AT1R activation is involved in the cascade of neuroinflammatory changes present after the SNL ligation in the spinal cord. Further experiments are needed to evaluate the possible use of losartan, an AT1R antagonist, as a possible neuropathic pain treatment.
This work was supported within the project The Centre of Biomedical Research (CZ.1.07/2.3.00/30.0025). This project is co-funded by the European Social Fund and the state budget of the Czech Republic.
Grant **Support:** CZ.1.07/2.3.00/30.0025, GACR305/09/1228, P304/12/G069, LH12058, RVO67985823

Disclosures: M.S. Diallo: None. **N. Kalynovska:** None. **J. Palecek:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.09/UU24

Topic: D.08. Pain

Title: Chronic neuropathic pain provokes adaptive changes in the response of endogenous pain inhibition in rats

Authors: *A. PARENT[1], P. TETREAULT[1], M. ROUX[1], K. BELLEVILLE[1], C. AURAY-BLAIS[2], P. GOFFAUX[3], P. SARRET[1];
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Abstract: As chronic pain sets in, physiological responses to tonic experimental pain may be altered, providing important clues to the development of chronic pain and to its neurobiological underpinnings. The underlying mechanisms leading to the development of chronic pain may also differ depending on the type of chronic pain (e.g. inflammatory or neuropathic). In order to better understand the pathological changes associated with the development of chronic pain, the formalin test was performed in separate groups of male SD rats, 12, 28 and 90 days after induction of chronic neuropathic or inflammatory pain. Central (CSF) and peripheral (plasma) serotoninergic (5-HTergic) activity was also measured immediately after the formalin test.
The hind paw complete Freund's adjuvant (CFA) model and the chronic constriction injury (CCI) model were used to induce chronic inflammatory and neuropathic pain, respectively. At each endpoint (days 12, 28 or 90), responses to tonic experimental pain were evaluated by injecting 50 µl of formalin 5% in the plantar surface of the contralateral hind paw in control and CFA/CCI animals. CSF and plasma samples were collected immediately after the formalin test, and the 5-HTergic activity was assessed using mass spectrometry.
In both models, we first highlighted the development of chronic pain by assessing mechanical withdrawal thresholds over time. We next observed a trend towards decreased pain-like behaviors during the formalin test among the neuropathic pain group on **day 12**. On **day 28**, a significant 55% decrease in pain-like behaviors (all ps<0.01) was measured during the acute and inflammatory phases of the formalin test in CCI rats. Interestingly, this decrease in pain-like behaviors was completely reversed on **day 90** (all ps>0.05). In sharp contrast, CFA-treated rats showed no apparent changes in their response to formalin. Moreover, we demonstrated that the formalin test triggered changes in the activity of the 5-HTergic system that may explain temporal

variations in behavioral responses in the different pain paradigms.
Our data reinforce the idea that chronic pain develops through diverse pathological mechanisms, depending on the origin of pain (neuropathic vs inflammatory). The decrease in pain-associated behaviors in CCI rats (on day 28) during the formalin test suggests that active pain inhibitory mechanisms and/or central desensitization are present in the early days of chronic pain. These processes may be part of a transitory adaptive process occurring at an intermediate developmental stage of chronic neuropathic pain.
AP holds a PhD scholarship from FRQ-S and CAN

Disclosures: A. Parent: None. **P. Tetreault:** None. **M. Roux:** None. **K. Belleville:** None. **C. Auray-Blais:** None. **P. Goffaux:** None. **P. Sarret:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.10/UU25

Topic: D.08. Pain

Support: Minnesota Medical Foundation

Viking's Children Fund

University of Minnesota, Center for Neurobehavioral Seed Grant

Title: Tmem35 (tuf1): A novel factor in pain pathway?

Authors: B. C. KENNEDY[1], J. G. DIMOVA[2], K. H. REISE[3], P. MARELL[3], W. VON HOHENBERG[2], J. C. GEWIRTZ[2], *P. V. TRAN[3];
[1]Program in Neurosci., [2]Dept. of Psychology, [3]Dept. of Pediatrics, Univ. of Minnesota, Minneapolis, MN

Abstract: Chronic pain is a major health problem, affecting over 100 million adults annually in the USA, with considerable economic costs in healthcare and loss of productivity. Notable progress has been made in elucidating neural mechanisms underlying pain sensing and processing, leading to important insights into the maladaptive changes that produce persistent or chronic pain. Moreover, susceptibility to such maladaptive changes depends in part on the individual's genetic makeup as well as the interaction between genes and environment. There are on-going efforts to identify molecules or genes that mark specific neuronal cell types and their respective roles in pain behavior to better understand at the molecular level the transition from

acute to chronic pain and to provide essential tools for tailoring effective pain treatments. Here, we propose the novel TMEM35 (TUF1) is one such candidate. Our group investigates a 167-aa polypeptide (TMEM35/TUF1) that is strongly expressed in developing and adult neural and endocrine networks that mediate pain (i.e., skin, dorsal root ganglia, spinal cord, brain stem, amygdala, hippocampus, cortex, and HPA axis). The involvement of TUF1 in pain was indicated by evidence of thermal hyperalgesia and mechanical allodynia in the tuf1 KO mice. In addition, there is a strong link between chronic pain and anxiety. The tuf1 KO mice exhibit increased anxiety-like behavior accompanied by higher basal level of plasma corticosterone and adrenal hyperplasia, providing additional rationale for assessing TUF1's role in pain pathways. Current experiments are investigating whether there are interactions between the stress and pain perception or whether these are independent effects of tuf1 gene deletion.

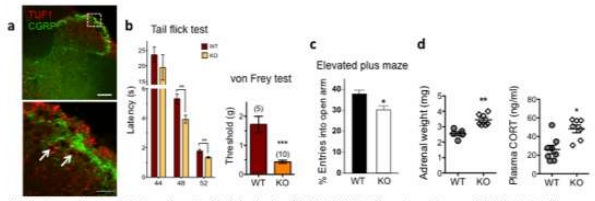

*Fig. 1: Increased pain sensitivity and anxiety-like behavior in tuf1 KO. **a)** CGRP+ fibers (green) innervate TUF1+ (red) cells (arrows in lower panel, scale bar = 20 μm) in adult rat dorsal cord lamina I. **b)** Tail-flick responses and paw-withdrawal tests (von Frey microfilaments) show lower pain thresholds in KO mice, WT=9, KO=10 at 48C, n=7/genotype at 52C. **c)** Anxiety-like behavior tested by elevated-plus maze shows KO made fewer entries into the open arms (WT=12, KO=6). **d)** Adrenal weight and basal plasma corticosterone (CORT) level were higher in KO mice. Unpaired t-test, *P<0.05, **P<0.01, ***P<0.001.*

Disclosures: B.C. Kennedy: None. **J.G. Dimova:** None. **K.H. Reise:** None. **P. Marell:** None. **W. Von Hohenberg:** None. **J.C. Gewirtz:** None. **P.V. Tran:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

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Topic: D.08. Pain

Support: NIH Grant NS061241

NIH Grant NS051336

Painless Research Foundation

Title: Increased responses to glutamate in dorsal root ganglion (DRG) neurons after peripheral nerve injury

Authors: *K. GONG[1], A. BHARGAVA[2], L.-H. KUNG[3], P. T. OHARA[3], L. JASMIN[3];
[1]Dept. of anatomy, [2]Dept. of Surgery, [3]Dept. of Anat., Univ. of California San Francisco, San Francisco, CA

Abstract:
Recent evidence supports the idea that glutamate in the periphery is involved in nociception. Using patch clamp we recorded neuronal
responses in dorsal root ganglia (*ex-vivo* DRG preparation). Following CCI, small DRG neurons (<30μm) exhibited an increased excitability associated with a decrease in the membrane threshold and Rhoebase.
Puff application of glutamate or of the selective ionotropic receptor agonists AMPA, KA, and the group I metabotropic receptor (mGluR) agonist DHPG, induced larger inward currents compared to naïve DRG. NMDA receptor mediated currents were unchanged. Additionally, we found that after CCI more neurons responded to glutamate, AMPA, DHPG as well as to NMDA, but not to KA. We also investigated the trafficking of glutamate receptors in the DRG. Following CCI there was a redistribution of NMDA receptors to the neuronal membrane while proportion of KA at the surface vs. in cytosol remained unchanged. We then examined the effect of group I mGluR activation on neuronal excitability and glutamate receptor responses. In naive rats, incubation of the whole ganglion in DHPG (50 μM) for 2 hours resulted in decreased membrane threshold and larger inward currents following KA application but smaller responses to AMPA and NMDA. In the CCI group, DHPG incubation increased neuronal rhoebase and decreased AMPA and NMDA induced currents. KA induced currents were unchanged. These results show that following nerve injury DRG neurons have increased excitability and larger inward currents to glutamate application. In addition, activation of Group I mGluR modulates the activity of ionotropic glutamate receptors differentially in naïve vs. nerve injured DRG neurons. We propose that peripheral glutamatergic transmission is involved in the initiation of neuropathic pain.

Disclosures: A. Bhargava: None. **L. Kung:** None. **P.T. Ohara:** None. **K. Gong:** None. **L. Jasmin:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.12/VV1

Topic: D.08. Pain

Support: NIH Grant DA27690

Title: Peripheral nerve injury reduces efficacy of gabapentin on neurotransmitter release in the prefrontal cortex and locus coeruleus

Authors: *T. SUTO, J. C. EISENACH, K. HAYASHIDA;
Anesthesiology, Pain Mechanism Lab., Wake Forest Univ. Shcool of Med., Winston-Salem, NC

Abstract: We have previously demonstrated that the antiepileptic drug gabapentin activates noradrenergic neurons in the locus coeruleus (LC) via glutamate-dependent mechanisms and that gabapentin induces a similar degree of spinal noradrenaline release in normal and L5-L6 spinal nerve ligated (SNL) rats. The current study examined whether SNL alters effects of gabapentin on noradrenaline release in the prefrontal cortex (PFC) and glutamate release in the LC following systemic or local gabapentin administration. We also examined attention-related behavior following systemic injection of gabapentin in normal and SNL rats.
Right L5-L6 SNL was performed in male Sprague-Dawley rats. Extracellular concentrations of noradrenaline in the PFC and glutamate in the LC ipsilateral to SNL were measured by microdialysis in behaving rats after SNL surgery, following either local perfusion into the PFC or intraperitoneal (i.p.) injection of gabapentin. Attention-related behavior was examined 1 hour after i.p. injection of gabapentin by measuring the time that animal showed physical contacts with the newly placed object in his cage.
In normal animals, PFC-perfused gabapentin concentration-dependently reduced the extracellular concentration of noradrenaline in the PFC, while systemically injected gabapentin dose-dependently increased extracellular concentration of noradrenaline in the PFC, associated with the increase in extracellular concentration of glutamate in the LC. In SNL animals, both local and systemic effects of gabapentin on noradrenaline release in the PFC and glutamate release in the LC were reduced compared to normal animals. SNL rats showed less attention-related behavior compared to normal animals and gabapentin did not affect attention-related behavior in either normal or SNL animals. These results indicate that peripheral nerve injury reduces efficacy of gabapentin on noradrenaline release in the PFC as well as glutamate release in the LC in behaving rats.

Disclosures: T. Suto: None. **J.C. Eisenach:** None. **K. Hayashida:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.13/VV2

Topic: D.08. Pain

Support: Swedish Research Council (04X-2887)

Karolinska Institutet funds

The Peter and Patricia Gruber Foundation

The China Scholarship Council

Title: Somatostatin and its 2A receptor in dorsal root ganglia and dorsal horn of mice and man: Expression, trafficking and possible role in pain

Authors: *T. SHI[1,2], M.-D. ZHANG[3], Q. XIANG[2], S. BARDE[3], K. FRIED[1], S. SCHULZ[4], T. HÖKFELT[3];
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Abstract: Anti-nociceptive effects of somatostatin (SST) and its receptor subtypes have been reported. We now demonstrate, using immunohistochemistry and *in situ* hybridization, presence of SST and SST receptor 2A (sst2A) protein and transcript in mouse and human dorsal root ganglia (DRGs) and spinal cord. sst2A immunoreactive (IR) neurons were found in mouse and human DRGs, the receptor protein always mainly cell membrane-associated and in mouse colocalized with CGRP (mostly) and/or NPYY1 receptor (sometimes) in small neurons. A high density of fibers was detected in lamina II in both mouse and human spinal dorsal horn. Dorsal root rhizotomy did not appear to abolish sst2A-immunoreactivity (LI) in the ipsilateral dorsal horn. Accumulation of sst2-LI was detected on the proximal and, more strongly, on the distal side of a sciatic nerve ligation. Systemic treatment of animals with the sst2 agonist octreotide (Oct) caused a dramatic internalization of sst2A in the DRG neurons, as also observed in dorsal horn and DRG neurons after intrathecal administration. Oct treatment attenuated the reduced pain threshold in a neuropathic pain model, in parallel suppressing the activation of p38 MAPK in the DRGs. These findings highlight a significant role of the SST system in pain signaling and suggest that sst2A may represent a novel target for pharmacological treatment of neuropathic pain.

Disclosures: T. Shi: None. **M. Zhang:** None. **Q. Xiang:** None. **S. Barde:** None. **K. Fried:** None. **S. Schulz:** None. **T. Hökfelt:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

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Topic: D.08. Pain

Support: Research grant D2 from Kansai Medical University (Y.S.)

Grants-in-Aid for Exploratory Research (M.S.), the Japan Society for the Promotion of Science

Scientific Research (B) (S.I.), the Japan Society for the Promotion of Science

Title: Pituitary adenylate-cyclase activating polypeptide (PACAP) is possibly regulated by RE1-silencing transcription factor (REST/NRSF) isoform in neuropathic pain

Authors: *Y. SHUDO, M. SHIMOJO, S. ITO;
Med. chemistry, Kansai Med. Univ., Hirakata/Osaka, Japan

Abstract: Neuropathic pain is one of the most common type of intractable pain which control is difficult, even if with morphine and detailed pathophysiological mechanism of neuropathic pain is still unknown. Our previous study reported that neuropeptide pituitary adenylate-cyclase activating polypeptide (PACAP) is required for the development of spinal sensitization and induction of neuropathic pain (Mabuchi *et al.*, J. Neurosci. 2004). PACAP is distributed widely in dorsal root ganglia (DRG) and increases after nerve injury. However detail mechanism remains unknown. It was reported that functional RE1-like element located in PACAP gene and the regulation of RE1 is controlled by the RE1-silencing transcription factor (REST/NRSF). Further studies reported that this RE1-like element contains many mismatches, resulting that *in silico* prediction identified the new RE1 site upstream of mouse PACAP gene. To analyze the regulation of PACAP gene by REST in neuropathic pain, we employed L5 sciatic nerve transection (L5-SNT) mouse model. The immunostaining and qRT-PCR analysis showed that PACAP dramatically increased in DRG in L5-SNT mice. REST4, a neuron-specific splicing isoform of REST also increased in DRG of L5-SNT mice, suggesting PACAP gene is regulated by REST4. To analyze the functional RE1 *in vitro*, we have cloned mouse PACAP promoter region with either wild or mutant RE1 into promoter-less luciferase vector. Reporter gene analysis performed with PC12 cells showed that wild-type RE1 suppressed promoter activity compared to mutant RE1. Over expression of REST4 in PC12 showed that PACAP expression increased. It was reported that novel protein nSR100 directly promotes alternative splicing of REST transcripts to produce REST4, thereby activating expression of REST targets in neural cells. In L5-SNT mice the expression of nSR100 increased higher corresponding to the expression of REST4. Taken together, these results suggested that the abnormal alternative splicing of REST producing REST4 by nSR100 might be involved in the regulatory mechanism of PACAP expression in neuropathic pain.

Disclosures: Y. Shudo: None. **M. Shimojo:** None. **S. Ito:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 267.15/VV4

Topic: D.08. Pain

Support: NIH-GM 102691

Title: Differential effects of natural rewards and pain on vesicular glutamate transporter (VGLUT) expression in the nucleus accumbens (NAc)

Authors: *M. LEE[1], D. TUKEY[1], D. XU[1], S. EBERLE[1], Y. GOFFER[2], E. ZIFF[1], J. WANG[1]; [1]NYU Sch. of Med., New York, NY; [2]Columbia Univ. Col. of Physicians and Surgeons, New York, NY

Abstract: Pain and natural rewards have both been shown to alter neuronal activities in the nucleus accumbens (NAc), a key component of the brain reward system, despite eliciting opposite behavioral consequences. Medium spiny neurons (MSNs) of NAc receive excitatory glutamatergic inputs and modulatory cholinergic and dopaminergic inputs from a variety of cortical and subcortical structures. In particular, glutamatergic projections from the prefrontal cortex, thalamus, amygdala, and hippocampus to the NAc provide distinct synaptic and ultimately, behavioral functions. The family of vesicular glutamate transporters (VGLUTs 1-3), which have distinct expression patterns throughout the brain, mediates the accumulation of glutamate into synaptic vesicles and plays a key role in glutamate transmission. In this study we use a spared nerve injury (SNI) model for chronic pain and a sucrose self-administration model for natural rewards in rats to examine the levels of VGLUTs (1-3) in the synaptoneurosomes of the NAc. We find that levels of VGLUT1 and VGLUT3 are decreased with chronic pain, and that VGLUT3 levels are increased with chronic administration of natural rewards. These results demonstrate that pain and natural rewards have distinct effects on VGLUT expression patterns in the NAc.

Disclosures: M. Lee: None. **D. Tukey:** None. **D. Xu:** None. **S. Eberle:** None. **Y. Goffer:** None. **E. Ziff:** None. **J. Wang:** None.

Poster

267. **Mechanisms of Neuropathic Pain: Neurotrophins and Neurotransmitters**

Location: Halls B-H

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Topic: D.08. Pain

Support: NIH Grant NS046606

NCI Grant CA 124787

Title: Activation of nerve growth factor-TrkA signaling in dorsal root ganglion in paclitaxel-induced peripheral neuropathy

Authors: *H. ZHANG[1], Y. LI[2], P. DOUGHERTY[2];
[1]Dept Anesthesiol, UT MD Anderson Cancer Ctr., HOUSTON, TX; [2]Pain Med., UT MD Anderson Cancer Ctr., Houston, TX

Abstract: Paclitaxel (Taxol®), a commonly used antineoplastic agent, causes peripheral neuropathy characterized by prominent impairment of touch sensations. Little is known about the mechanisms underlying paclitaxel-induced peripheral neuropathy. Although co-administration of neurotrophin such as nerve growth factor (NGF) has been reported to prevent peripheral neuropathy in preclinical studies, the therapeutic effect of such a treatment is not clear in clinical trials. The goal of this study is to determine the role of two neurotrophic factors, NGF and brain-derived neurotrophic factor (BDNF), in dorsal root ganglia (DRG) in the development of paclitaxel-induced peripheral neuropathy. Adult Sprague-Dawley rats (55-60 days) were treated with paclitaxel (2mg/kg, q2d x 4, i.p.) or vehicle and sacrificed 4 hours, 7 days, 16 days and 29 days after treatment. The L4 and L5 DRGs of both sides were collected for rtPCR and western blotting experiments. The mRNA level of NGF but not BDNF was increased at both 4 hours and 7 days following chemotherapy. Western blotting detected an increase of both NGF and its receptor TrkA 7 days following chemotherapy. The protein of BDNF was not detected in DRG while the expression of its receptor TrkB was not changed. Finally, intrathecal application of anti-NGF antibody before and during chemotherapy partially prevented paclitaxel-induced mechanical hypersensitivity but such a treatment did not reverse the established neuropathy induced by paclitaxel. The results suggest that the paclitaxel induces the activation of NGF-TrkA signaling in DRG which may contribute to the initiation but the not the maintenance of paclitaxel-induced peripheral neuropathy.

Disclosures: H. Zhang: None. **Y. Li:** None. **P. Dougherty:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.01/VV6

Topic: D.08. Pain

Support: American Fibromyalgia Syndrome Association (AFSA)

Intramural Research Program of the NIH, National Center for Complementary and Alternative Medicine

Title: Differential relationship between insular gray matter volume, pain sensitivity and cognitive performance in younger and older fibromyalgia patients

Authors: *M. CEKO[1,2], M. C. BUSHNELL[2], M.-A. FITZCHARLES[1], P. SCHWEINHARDT[1]; [1]McGill Univ., Montreal, QC, Canada; [2]NCCAM, NIH, Bethesda, MD

Abstract: We have previously reported insular gray matter (GM) increase in younger fibromyalgia (FM) patients (Ceko et al. 2012), which correlated with lower pain sensitivity, possibly indicating the recruitment of endogenous pain modulatory mechanisms. Here we investigate the effect of insular GM hypertrophy in these patients on the relationship between cognitive performance and pain sensitivity, and explore if age-related insular GM decline (Ceko et al. 2012) affects this relationship in older patients.

We measured cognitive performance and pain sensitivity in 14 younger FM patients compared to 15 controls (mean ages of 42.4 and 43.1, p=0.8), and 14 older patients compared to 13 controls (mean ages of 55.0 and 55.4, p=0.8), matched for handedness, education, physical activity, and socioeconomic status. All subjects underwent anatomical magnetic resonance imaging (MRI). Cognitive performance was assessed using the Auditory Consonant Trigram (ACT) memory task. Pain sensitivity was assessed in response to a standardized pressure stimulus applied on the thumbnail. Anatomical MRI data were preprocessed in SPM8. Group difference on the ACT were tested with an univariate ANOVA. Mean GM in areas of group differences was correlated pain sensitivity, as well as with the ACT score (difficult level), controlling for age.

Both groups of patients had cognitive impairment compared to matched controls. Cognitive performance was in younger patients inversely related to pain sensitivity. Younger patients with lowest pain sensitivity and best cognitive performance had most GM in the insula. In older patients, insular GM was no longer positively correlated with cognitive performance (or protective against pain sensitivity). Similarly, while negatively correlated in younger patients, cognitive performance and pain sensitivity were no longer negatively correlated in older patients. In younger patients, insular hypertrophy is related to decreased pain sensitivity and increased cognitive performance. As patients age insular GM declines and thus the positive effect on pain sensitivity and cognitive performance is lost.

Ceko et al. 2012 Neurosci Abstracts, Society for Neuroscience 2012.

Oosterman et al. 2013 Eur J Pain.

Disclosures: M. Ceko: None. **M.C. Bushnell:** None. **M. Fitzcharles:** None. **P. Schweinhardt:** None.

Poster

268. **Pain Imaging and Perception II**

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Program#/Poster#: 268.02/VV7

Topic: D.08. Pain

Support: Ministry of Internal Affairs and Communications entitled, 'Novel and innovative R&D making use of brain structures'

Title: Integration of pain and reward in decision making: An fMRI study

Authors: *M. MARUYAMA[1], W. YOSHIDA[1], S. ISHII[1,2], B. SEYMOUR[3,4];
[1]Advanced Telecommunications Res. Inst. Intl., Kyoto, Japan; [2]Integrated Systems Biol. Laboratory, Dept. of Systems Science, Grad. Sch. of Informatics, Kyoto Univ., Kyoto, Japan; [3]Ctr. for Information and Neural Networks, Natl. Inst. for Information and Communications Technol., Osaka, Japan; [4]Computat. and Biol. Learning Laboratory, Dept. of Engin., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Current theories of the motivational basis of pain highlight it's inhibitory relationship with reward. In particular, the Motivation-Decision model (1,2), and the Opponent Reinforcement model (3,4) predict a simple additive process between the positive value of rewards and the negative value of pain, with the net value determining motivational responses and decisions. Although this model explains a wealth of behavioural and neural data (5,6), recent imaging findings have suggested that reward and pain might not only sum, but interact (7) - finding that fits awkwardly with standard models. The current study used fMRI in healthy subjects to investigate how the brain integrates values of monetary reward and painful shock as gain and cost, respectively, to explore the nature of this interaction. Each trial started with an offer that was a pair of visual cues indicating a subjective pain intensity and an amount of monetary reward. The subjects were instructed to press one of two buttons immediately after they made a decision to accept or reject the offer. In one condition, when the offer was accepted, they received both the painful shock and money with a fifty percent probability. In a second condition, they received *either* the painful shock or the money. Comparison of the two conditions allowed evaluation of the nature of the potential interaction between decision outcomes. The delivery of painful shock occurred at the end of trials, and the total accumulated reward money was remunerated after the experiment. Although the choice behaviour of subjects was similar

between each condition, the reaction time was longer both when the net subjective value offer was close to zero (i.e. more decision conflict), and in the condition when the outcomes where presented as either – or alternatives compared to when they occurred together. To probe this further, we modeled the data using a hierarchical drift diffusion model (8,9) to provide a specific, mechanistic account of value integration. fMRI data will be presented that support the corresponding neural basis of the integration and decision process.

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Disclosures: M. Maruyama: None. **W. Yoshida:** None. **S. Ishii:** None. **B. Seymour:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.03/VV8

Topic: D.08. Pain

Title: Long-lasting effect of transcranial direct current stimulation (tDCS) in healthy subjects: An evoked potential study

Authors: *M. HUNG[1], J.-C. HSIEH[1,2], D. M. NIDDAM[1,2];
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Abstract:
Background: Anodal tDCS over primary motor cortex (M1) can reduce acute pain. However, relatively little is known about how to best prolong the modulatory effect while minimizing the number of tDCS sessions. Here we seek to test a novel "boost" paradigm in which stimulation is performed daily for 5 days in the first week and on the fifth day of the 2nd and 3rd week. Hypothesis: Repetitive tDCS stimulation using a "boost" paradigm can (1) elevate the acute pain

threshold (PT) and this effect can be maintained for up to 9 weeks post tDCS, and (2) modulation of the PT is reflected in cerebral activity as measured with contact heat evoked potentials (CHEPs). **Methods:** 48 healthy subjects received either anodal or sham tDCS according to a novel "boost" paradigm. Before and after each stimulus session, sensory and pain thresholds to warm thermal stimuli were recorded. In addition, CHEPs were recorded before and after the 1st, 5th, 6th and 7th tDCS sessions. The stimulus temperature used to evoke CHEPs was rated as moderately painful prior to the first tDCS session and was kept constant at subsequent CHEP recordings. To monitor the durability of the 7 tDCS sessions, sensory and pain thresholds were also recorded in response to thermal stimulation on day 5 in week 4, 5, 6, and 12 without tDCS stimulation. **Results:** Anodal tDCS substantially increased the thermal PT. The elevated pain thresholds were sustained up to 12 weeks after the initial tDCS and 9 weeks after the last tDCS session. The peak-to-peak CHEP amplitude at the vertex decreased on the fifth day of tDCS and this reduction was sustained until the 3rd week (7th tDCS session). **Conclusions:** Repetitive anodal tDCS over M1 delivered in "boosts" can maintain an elevated heat PT for up to 9 weeks post tDCS. Elevation of the PT was mirrored by reduced CHEP amplitudes. Taken together, both subjective and objective measures support a prolonged sustained effect in response to our "boost" paradigm. The tDCS protocol presented here is less time consuming than daily stimulation for several weeks and offers a reduced burden to possible patients.

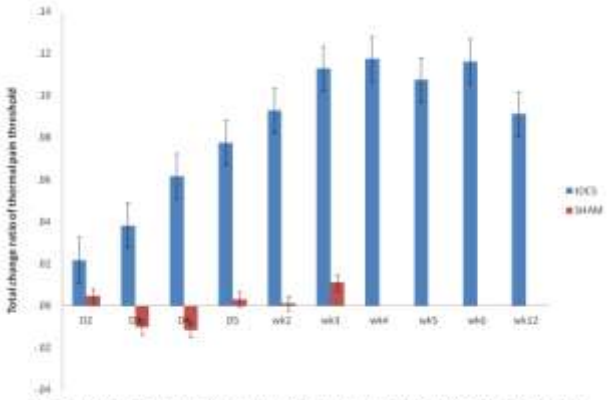

Fig. Significant differences between groups were found on day 3 (D3) to week 3(wk3)($p<0.001$). Significant within group differences were found in week 2 (wk2) to week 12 (wk12) ($p<0.01$). The standard error of the mean is indicated by bars.

Disclosures: M. Hung: None. **J. Hsieh:** None. **D.M. Niddam:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.04/VV9

Topic: D.08. Pain

Support: FRQ-S (Fonds de recherche du Québec – Santé)

Title: Neuronal activity in the precuneus and superior temporal gyrus during the initial experience of pain predicts exaggerated pain at recall

Authors: *L. LAGRANDEUR, J. PLOURDE-GAUTHIER, K. DAIGLE, G. LÉONARD, P. GOFFAUX;
Ctr. De Recherche Clinique (CRC), Ctr. Hospitalier Universitaire De Sherbrooke (CHUS), Fleurimont, QC, Canada

Abstract: Introduction:
Past pain is rarely recalled accurately, a phenomenon known as mnemonic pain bias. Although the existence of mnemonic pain bias is well documented in both clinical and experimental settings, the underlying neurophysiology is poorly understood. In the present experiment, we tested the possibility that mnemonic pain bias could be traced back to the functional brain activity evoked when pain is initially experienced (i.e., encoded).
Methods:
Thirteen healthy adults were exposed to painful transcutaneous sural nerve shocks. Shock-evoked cerebral activity was measured using somatosensory-evoked brain potentials (SEPs). SEPs were transformed from scalp recordings to intracranial current sources using standardized low-resolution electromagnetic tomography (sLORETA). Subjective pain intensity was assessed using a visual analog scale devoid of numerical anchors. Two months after having experienced sural nerve pain in the lab, participants were called back and asked to recall the intensity of the pain they had initially experienced. The difference between recalled pain and the pain initially felt was used as our metric of mnemonic pain bias. In our main analysis, correlations between pain-evoked brain activity and mnemonic pain bias were analysed using Pearson correlations corrected for multi-voxel comparisons (SnPM). This approach allowed us to identify the set of brain regions which predict individual differences in mnemonic pain bias.
Results:
Results revealed that mnemonic pain bias was negatively correlated with early, contralateral precuneus activity ($r=-0.89$, $p<.002$, activated 112-116ms after shock onset), and positively with late contralateral superior temporal gyrus activity ($r=0.90$, $p<0.007$, activated 428-432ms after shock onset). These structures were not part of the network of brain regions which predicted subjective pain intensity (which in our study included the supplementary motor area, the anterior cingulate cortex, and the insula).
Conclusions:
Exaggerated pain recall was associated with both decreased precuneus and increased superior temporal gyrus activity during then encoding process. This suggests that mnemonic pain bias involves brain regions important for both self-perception (precuneus) and memory formation (superior temporal gyrus).

Disclosures: L. Lagrandeur: None. **J. Plourde-Gauthier:** None. **K. Daigle:** None. **G. Léonard:** None. **P. Goffaux:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.05/VV10

Topic: D.08. Pain

Support: R01-AT004714

P01-AT002048

P01-AT006663

R01-AT005280

R01-AG034982

R21-AR057920

Title: Disrupted brain circuitry for reward/punishment in fibromyalgia

Authors: *M. L. LOGGIA[1], C. BERNA[3], J. KIM[2], C. CAHALAN[4], R. L. GOLLUB[5], A. D. WASAN[4], R. E. HARRIS[7], R. R. EDWARDS[4], V. NAPADOW[6];
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Abstract: While patients suffering from fibromyalgia (FM) are known to exhibit hyperalgesia, the central mechanisms contributing to this altered pain processing are not fully understood. In this study we investigated potential dysregulation of the neural circuitry underlying cognitive and hedonic aspects of the subjective experience of pain such as anticipation of pain and anticipation of pain relief. We adopted a region-of-interest (ROI) approach focused on the nucleus accumbens (NAc) and the ventral tegmental area (VTA), two mesolimbic structures known to be involved in the processing of reward and punishment, and that were implicated in FM pathophysiology in PET studies.
BOLD FMRI (Siemens TIM Trio, 3T, TR/TE=2sec/30ms, voxel size=3.1x3.1x4mm) was

performed on 31 FM patients and 14 controls while they received cuff pressure pain stimuli on their leg, calibrated to elicit a pain rating of ~50/100. During the scan, subjects also received visual cues informing them of impending pain onset (pain anticipation) and pain offset (relief anticipation). Patients exhibited less robust activations during both anticipation of pain and anticipation of relief within regions commonly thought to be involved in sensory, affective, cognitive and pain-modulatory processes. In healthy controls, a direct search in the ventral tegmental area (VTA) revealed a pattern of activity compatible with the encoding of punishment: activation during pain anticipation and pain stimulation, but deactivation during relief anticipation. Furthermore, in these subjects, VTA responses to anticipation of pain and relief anticipation were negatively correlated ($r = -0.76$, $p = 0.002$). In FM patients, however, VTA activity during pain and anticipation (of both pain and relief) periods was dramatically reduced or abolished. Moreover, VTA responses to pain anticipation and relief anticipation were not significantly correlated in the FM patients ($r = -0.12$, $p = 0.52$). No group differences were observed for the NAc.
Our results suggest that FM patients exhibit disrupted brain responses to reward/punishment. The VTA is a source for reward-linked dopaminergic/GABAergic neurotransmission in the brain and our observations are compatible with reports of altered dopaminergic/GABAergic neurotransmission in FM. Disruption of these pathways may relate to the augmented central processing of pain and reduced efficacy of opioid treatments in FM patients.

Disclosures: M.L. Loggia: None. **C. Berna:** None. **J. Kim:** None. **C. Cahalan:** None. **R.L. Gollub:** None. **A.D. Wasan:** None. **R.E. Harris:** None. **R.R. Edwards:** None. **V. Napadow:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.06/VV11

Topic: D.08. Pain

Support: Pfizer Inc. Study number A0081211

Title: Further evidence for sensory hypersensitivity in fibromyalgia: Sensitivity to visual stimuli and response to pregabalin

Authors: E. ICHESCO[1], A. KAIRYS[1], E. CHANG[2], G. RAMIREZ[1], D. J. CLAUW[1], R. E. HARRIS[1], *S. E. HARTE[1];
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Abstract: INTRODUCTION: Fibromyalgia (FM) is a chronic disorder characterized by widespread pain and muscle tenderness. Additionally, FM patients report hypersensitivity to non-somatic sensory stimuli including auditory and olfactory stimulation, suggesting a global central nervous system state of sensory amplification. Here we examined the enhanced sensitivity to experimental visual stimuli in FM.
METHODS: Nine female FM patients underwent two presentations of a 3 minute visual stimulation task, consisting of an alternating series of flashing blue-yellow annulus checkerboard images displayed at varying intensities of illuminance (5 lux - 75 lux) and a static fixation cross (control image). Subjects rated each image and the entire task on a 21-box numerical descriptor scale of sensory unpleasantness. These results of quantitative sensory testing were compared with results in 11 age- and sex-matched healthy controls (HC). An independent samples t-test was performed using SPSS to compare overall unpleasantness ratings between the FM and HC groups. In a separate sample of 17 FM participants, a 3 minute fMRI scan was conducted with the presentation of the same flashing checkerboard visual stimulus. Data were preprocessed in SPM5 and single subject results were contrasted between the flashing checkerboard and static fixation blocks. A one-sample t-test was performed using age as a regressor, and results deemed significant at $p < 0.05$ corrected at the cluster level with an uncorrected voxel extent threshold of $p < 0.001$.
RESULTS: FM patients (mean = 9.9, SD = 6.2) rated the visual stimulus task to be significantly more unpleasant compared to HCs (mean = 2.9, SD = 3.3; $p < 0.01$). When the visual task was presented to patients during fMRI, in addition to the expected activation of the visual cortex, significant activations were observed bilaterally in the anterior insula (right: z = 4.73, cluster level corrected p = 1.89 x 10-7; left: z = 4.65, cluster level corrected p = 1.99 x 10-7). In a previously presented study, we showed that this effect was significantly attenuated by administration of pregabalin, but not placebo.
CONCLUSION: Preliminary analysis indicates FM patients experience greater unpleasantness to a dynamic visual stimulus compared to healthy controls suggesting a global, centrally-mediated sensory hypersensitivity in this chronic pain population, and that this phenomenon is attenuated by pregabalin administration. Additional studies are needed to replicate these findings and compare neuroimaging data between other cohorts of chronic pain patients and healthy controls.

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relationship even if those funds come to an institution.; Forest Laboratories, Inc., Merck & Co., Inc.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.07/VV12

Topic: D.08. Pain

Title: Inverted medial prefrontal response during pain facial expression in chronic pain patients

Authors: *E. VACHON-PRESSEAU[1], M. ROY[2], M. KUNZ[4], M.-O. MARTEL[5], M. J. SULLIVAN[6], T. D. WAGER[3], P. L. JACKSON[7], P. RAINVILLE[8];
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Abstract: Current models of pain expressivity suggest that individuals are born with the natural propensity to express pain and gradually learn to down-regulate these behavioral manifestations following the development of prefrontal inhibitory mechanisms. The regulation of facial pain expressiveness is also thought to be shaped by associative learning processes related to persistent pain and contextual factors. Specifically, facial expressions of pain may become adapted to elicit solicitous behavior. The aim of the present study was to determine if the brain networks regulating facial expressiveness are modified in chronic back pain (CBP) patients. To test this, we combined functional magnetic resonance imaging (fMRI) with in-scanner video recording of spontaneous facial expressions of pain. Pain was elicited by individually adjusted heat pain delivered to the legs of 14 CBP patients and 16 healthy participants. Facial expression intensity was quantified with the facial action coding system (FACS). This allowed us to identify brain regions whose activity varied parametrically with trial-to-trial variation in facial expressiveness and pain ratings, holding constant stimulus intensity. Multilevel linear modeling revealed that CBP and healthy participants differed in the strength of the relationship between pain intensity ratings and facial expressions ($p = .05$): a moderate but significant relationship was observed in the healthy participants ($b = .30$ $p = .01$), but not in the CBP patients ($p=0.84$). Brain activity in the leg region of somatosensory cortex covaried with pain intensity in both groups. Brain activity in the medial prefrontal cortex (mPFC) tracked facial expressiveness in a group-dependent manner. Replicating previous findings, the mPFC was inversely related to pain expressiveness in healthy individuals, consistent with a role in the inhibition of facial expression. However, this

effect was reversed in CBP patients, who displayed more mPFC activity in trials involving stronger facial responses. These findings are consistent with recent studies showing that, as pain becomes chronic, the mPFC shifts from being negatively to positively associate with pain. Furthermore, this shift may reflect associative learning processes, in which acute pain expressivity in CBP patients reflect the spontaneous integration of additional socio-contextual factors associated with chronicity and involving the activation of prefrontal circuits. This may provide a neural mechanism underlying the commonly observed dissociation between self-report and spontaneous behavioral manifestations of pain in chronic pain patients.

Disclosures: E. Vachon-Presseau: None. **M. Roy:** None. **M. Kunz:** None. **M. Martel:** None. **M.J. Sullivan:** None. **T.D. Wager:** None. **P.L. Jackson:** None. **P. Rainville:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.08/VV13

Topic: D.08. Pain

Title: Common representation of motor and pain processing in human cerebellum

Authors: *G. MISRA[1], S. A. COOMBES[2];
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Abstract: The human cerebellum has traditionally been considered a motor area. Recent evidence has shown that the cerebellum is also engaged during the experience of acute pain. Although lobules V and VI in the cerebellum are considered sensorimotor regions that are recruited for both motor and pain related processing, cerebellar activity has not been examined in the same group of individuals in the context of motor and pain processing. In the current study we used functional magnetic resonance imaging (fMRI) to examine which cerebellar regions are commonly active during separate instances of motor processing and pain processing. fMRI data was recorded from fifteen healthy right-handed individuals during two separate conditions: a) performance of a visually guided grip-force task with the right hand, and b) pain-eliciting thermal stimulation delivered to the thenar eminence of the right hand. Similar visual feedback was provided in the two conditions. The SUIT toolbox of Statistical Parametric Mapping (SPM) software was used to isolate the cerebellum from the anatomical and functional scans, and to align them to the SUIT-template of the cerebellum. Analysis of Functional NeuroImages (AFNI) software was used for data-preprocessing and statistical analysis. We demonstrate for the first time spatially overlapping neural activity for motor and pain processing in the cerebellum in the

same group of individuals. Overlapping activity was found in four cerebellar regions: bilateral lobules V, VI, VIIb and Crus II, which suggests existence of common neural substrate for motor and pain processing in the cerebellum. Lobules V and VI have previously been implicated in sensorimotor functions and we extend these findings by showing overlap of activity in these lobules during motor and pain processing. Previous observations suggest that the overlap in Crus II and VIIb may reflect more general processes such as visuospatial processing and executive function that were common to both tasks. Identification of a common substrate for motor and pain processing in the cerebellum has ramifications for our understanding of the neural circuits that govern the effects of pain on motor function and vice versa.

Disclosures: G. Misra: None. **S.A. Coombes:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.09/VV14

Topic: D.08. Pain

Support: Wellcome Trust Fellowship

Title: Does the PAG mediate distraction-based analgesia?

Authors: W. E. RUSSELL[1], *A. E. PICKERING[2], J. BROOKS[3];
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Abstract: Modulation of pain sensation through attention/distraction processes is a robust phenomenon (Villemure (2002). PAIN 95:195-199). To date, only a single study has demonstrated that PAG activity is correlated with the degree of analgesia during self-generated distraction from pain (Tracey (2002). J Neurosci 22: 2748-52). Therefore we investigated the relationships between hindbrain and cortical activity during a rigorously controlled visual distraction paradigm co-administered with painful stimulation.
In 20 healthy controls, activity in response to thermal heat stimuli (applied to the left arm using CHEPS pathway) and a simultaneous rapid serial visual presentation (RSVP) task was recorded using BOLD-sensitive fMRI at 3T. A 2x2 (+1) design was used, with thermal stimulus type (painful vs innocuous), a distraction task (hard vs easy) and a control condition with pain and minimal task load.
Functional images were corrected for local magnetic field distortions using fieldmaps, and registered to the MNI152 template. A repeated measures ANOVA was performed using a mixed effects model in FEAT v6. Data are reported for $Z>3.09$ and cluster corrected $p<0.05$.

An RM-ANOVA showed a main effect of temperature on pain scores and a task x temperature interaction ($P<0.001$). Post-hoc paired t-test revealed an effect of task only in the high temperature condition ($P<0.007$).
Group analysis demonstrated an expected main effect of pain in several cortical and sub-cortical areas (R. & L. anterior insula, R. posterior insula (PI), R. S1 and R. thalamus) and a negative main effect in peri-genual cingulate (PGC). For the visual attention task there was a positive main effect bilaterally in occipital lobes (primary visual cortices), parietal lobes, anterior insulae, and right superior/middle frontal gyrus. No areas showed a significant interaction.
Region of interest (ROI) analysis of PI, anterior cingulate, rostromedial medulla, PAG, S1, thalamus and PGC region was undertaken using functional masks. A positive correlation was found between PAG activity and the analgesic effect in the control versus hard task ($r2=0.22$, $P<0.05$) and a related negative correlation was found between PGC activation under the same condition ($P=0.05$, $R2=0.19$). Additionally a negative correlation was found between the control pain score and PAG activity ($r2=-0.24$, $P=0.03$). No other region showed significant correlations. This confirms and extends previous findings (Brooks et al, SfN 2012) and others that increased activity in the PAG positively correlated with distraction analgesia and identify it as an important locus in determining both analgesic effects and overall pain intensity.

Disclosures: W.E. Russell: None. **J. Brooks:** None. **A.E. Pickering:** None.

Poster

268. **Pain Imaging and Perception II**

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Program#/Poster#: 268.10/VV15

Topic: D.08. Pain

Support: SZSITIC JC201005270293A

CAS Bairen Fellowship

NSFC 31200856

Title: The effect of placebo on the modulations of spontaneous oscillatory activity to tonic muscle pain

Authors: *L. LI[1], L. HU[2], H. WANG[1], X. KE[3], Y. YUAN[1], X. LIU[1], Y. QIU[1];
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Southwest Univ., Chongqing, Sichuan, China; [3]Dept. of Pain, 2nd Clin. Med. Col. of Jinan University, Shenzhen People's Hosp., Shenzhen, Guangdong, China

Abstract: Placebo analgesia has been previously studied using LEP, which were decreased along with the reduction of pain perception. However, only the placebo affected early nociceptive processing to influence the pain perception was captured by LEP, and the later contribution from placebo (e.g., pain evaluation) to the pain perception reduction cannot be assessed using the duration-limited LEP, which were triggered by phasic laser stimuli. Here, we hypothesize that a comprehensive exploration of placebo affected nociceptive processing could be assessed using the changes of both pain perception and EEG responses that induced by prolonged noxious stimuli. Here we applied an experiment design consisting of four order-fixed and prolonged innoxious/noxious stimuli: (a) pain anticipation, (b) pain, (c) pain anticipation with placebo, (d) pain with placebo. For pain anticipation, isotonic saline (0.9%, 75 μl/min) were infused into the right masseter muscle of the subjects. For pain, hypertonic saline (5%) were infused into the left masseter muscle, and the speed of infusion was adjusted using a computer-controlled closed-loop system based on the real-time feedback of pain perception to make sure the relative stability of the perceived pain. In conditions c & d, the placebo was introduced by infusion of isotonic saline (0.9%) into the antecubital intravenous port in the right upper limb, while they were instructed by infusion of Entacapone which might have analgesic effect. EEG data were collected using 128 electrodes from 19 healthy subjects (3 females), aged 23 ± 2. The recorded EEG data were re-referenced to a common average reference. After excluding 5 non-responders, significant reduction of pain perception at condition d than at condition b was observed from the remaining 14 subjects ($p = 0.013$). Spectral analysis and two-way repeated measures ANOVA of alpha oscillatory amplitudes (factor1: without vs with placebo; factor2: pain anticipation vs pain) revealed a significant interaction at central-frontal electrodes (near Cz) ($F = 13.61$, $p = 0.003$). Post hoc Tukey's test revealed significant increase of amplitude of alpha oscillation in pain with placebo compared with pain. Prior to placebo instruction, significant correlations were observed between intensity of pain perception and the amplitude of alpha oscillation (at Cz) in conditions a ($R = 0.385^{**}$) and b ($R = -0.381^{**}$). No significant correlation was observed in conditions c & d. Placebo modulation of pain perception to prolonged noxious stimuli could be reflected by the changes of alpha oscillation at central-frontal area, which could serve as a physiologic marker of placebo analgesia during tonic pain.

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Poster

268. **Pain Imaging and Perception II**

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Program#/Poster#: 268.11/VV16

Topic: D.08. Pain

Support: DFG Grant PL 321/10-1

DFG Grant RTG 1373

Else Kröner-Fresenius-Stiftung Grant 2011_A82

Title: Neurophysiological correlates of tonic pain

Authors: E. SCHULZ, L. TIEMANN, M. POSTORINO, *M. PLONER;
TU Muenchen, Munich, Germany

Abstract: Recent functional imaging and neurophysiological studies have significantly extended our knowledge about the cerebral processing of pain. However, most of these results originate from experimental studies using brief painful stimuli. Whether and how these findings translate to the main clinical problem of tonic and chronic pain is largely unknown yet.
Here, we used EEG to explore neuronal activity that code for the intensity of long-lasting tonic pain. During the recording of a 64 channel EEG, we applied tonic heat pain and phasic laser pain in two counterbalanced experiments to healthy human subjects. The intensity of the stimuli was adapted to the subjects' individual pain level (analogue scale: 30 to 70, max. 100). Our results confirm recent findings showing that the intensity of phasic pain is related to neuronal theta, alpha and gamma activity. Moreover, our results reveal that neuronal activity at theta, beta and gamma frequencies with distinct topographies code for the intensity of long-lasting tonic pain. Interestingly, these topographies differ from those of phasic pain.
Taken together, the present investigation shows an ecologically more valid representation of the perception of pain in the human brain. It supports other evidence that a number of neuronal processes, brain regions and brain responses (such as gamma and theta activity) contribute to the highly subjective experience of pain.

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Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

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Program#/Poster#: 268.12/VV17

Topic: D.08. Pain

Support: NIH NS069909-01

Dana Foundation

Title: Functional MRI mapping of nociceptive cold and tactile activations in somatosensory areas in anesthetized monkeys

Authors: *P.-F. YANG[1,2], L. CHEN[1,2];
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Abstract: Cortical responses to innocuous tactile and nociceptive cold stimuli in early somatosensory areas along the central and the lateral sulci were examined by using high-resolution fMRI in anesthetized squirrel monkeys. All scans were performed on a 9.4 T Varian magnet, using a 3 cm surface transmit-receive coil positioned over the somatosensory cortices contralateral to the stimulated hand. For mapping tactile responses, we used 8 Hz vibrotactile stimulation (in 30 s on/off blocks) of individual distal finger pads. For mapping nociceptive cold responses, we stimulated the glabrous skin of fingers, via a Medoc ATS probe, with temperatures ranging from innocuous (15 $^{\circ}$C) to noxious (4 and 7 $^{\circ}$C) levels (in 21 s on and 30 s off blocks). FMRI data was acquired using a gradient echo planar imaging (GE-EPI) sequence (TE=16 ms; TR=1.5 s; 0.575 X 0.575 X 2 mm^3 or 0.275 X 0.275 X 2 mm^3 resolution). After 2D motion correction, all functional data were analyzed with AFNI. Within each animal, the nociceptive cold responses were reproducible across imaging runs. Noxious cold stimulation of digits evoked multiple spatially distinct BOLD activations in areas 3a, 3b, 1 and secondary somatosensory cortex (S2), whereas less frequent activation was present in M1. Tactile stimulation of digits evoked multiple spatially distinct and focal BOLD activations in area 3b, 1 and S2. In general, noxious cold and tactile activations did no overlap in area 3b. The spatial relationship between nociceptive cold and tactile activations in area 3a, 1 and S2 and the quantification of temperature depended BOLD signal changes are under investigation.

Disclosures: P. Yang: None. **L. Chen:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.13/VV18

Topic: D.08. Pain

Title: Differences in cortical activation patterns during pain with short and long durations and the effect of mental stress

Authors: *R. RINGLER[1], P. V.D. KEYLEN[2], K. DETMAR[4], R. LOOSE[4], C. FORSTER[3];
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Abstract: Introduction:
In this study mental stress was induced by a false color stroop pattern to decrease the pain perception in subjects during cold pressure test (CP). The modulation and cortical representation of pain processing during short term CP (stCP) and long term CP (ltCP) stimuli was studied using psychophysical methods and BOLD-fMRI. Stroop (ST) was then used for stCP and ltCP to further discriminate the effect of mental stress.
Materials and methods:
14 healthy subjects (3 m/11 f, mean age 23.4y±2.9y) participated. Ethics committee approval was granted. In a first experiment psychophysical data (rating, blood pressure, heart rate) were recorded. In the second experiment fMRI was assessed using a block design with alternating stimulus and baseline of different time intervals. Short term: ST 30s and stCP, stCP+ST 60s. Long term: ltCP and ltCP+ST 120s. All stimuli ST, CP or CP+ST were alternately repeated 2 times applied with baselines in between. During baseline and ST the right foot was surrounded by “warm” water at 14°C (57°F) while under CP and CPS by “cold” water at 4°C (40°F). The Stroop task used was a false color task. fMRI data were acquired on a 1.5 T Siemens. All data were normalized to Talairach and transformed to a MPRAGE data set. fMRI was analyzed using a group study GLM design with BrainVoyager QX
Results:
The psychophysical studies and the fMRI showed significant lower pain ratings during CP+ST as compared with CP. Blood pressure was increased during the painful stimuli of CP.During the stCP positive BOLD activation clusters were of smaller size (number of activated voxels) and a lower correlation value compared to long term cold pressure. Only during the stCP negative BOLD clusters were found in BA 4 (precent. Gyrus), BA 6 (Middle Frontal Gyrus, MFG) and BA 8 (Sup. Front Gyrus) but not during the ltCP. Both stimuli lead to positive BOLD clusters in pain specific areas such as ACC, BA 44 and in the Anterior Insula on both hemispheres (only for ltCP) and medial Insula for ltCP and stCP.The effect of the stroop task on the ltCP and stCP induced activations was tested by a contrast analysis.
Conclusion:
Mental stress as it is induced by a stroop task effectively reduced the pain ratings of both long term and short term cold pressure tests. The changes of the BOLD signals of many pain related brain areas were more pronounced during the long term stimulus indicating a stronger integration of the affected areas with increasing pain duration. Negative BOLD changes in mid frontal and orbito frontal regions during short term cold pressure stimuli can be interpreted as a sign of deactivations due to a reduced emotional rating of these pain stimuli which are known to be only short in duration.

Disclosures: R. Ringler: None. **P. V.D. Keylen:** None. **C. Forster:** None. **K. Detmar:** None. **R. Loose:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.14/VV19

Topic: D.08. Pain

Support: MSU Department of Family Medicine: Pearl Aldrich Graduate Fellowship

Title: Increased acute pain behaviors in severe Alzheimer's patients are associated with reduced functional connectivity to sensory and modulatory pain structures

Authors: *P. A. BEACH[1], M. MIRANDA[2], K. SWANIC[2], L. L. SYMONDS[3], D. C. ZHU[4], A. BOZOKI[5];
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Abstract: Though recent studies suggest increased acute pain sensitivity in Alzheimer's disease (AD) patients an associated neural mechanism has not been elucidated. Further, few studies have included patients with severe AD (sAD). Integrating behavioral pain testing with functional neuroimaging is a novel avenue for determining such a mechanism. Studies of normal subjects and pain patients suggests altered resting dynamics involving pain modulatory regions is correlated with enhanced acute pain. We predicted behavioral signs of increased pain sensitivity in AD would be associated with dysfunctional brain connectivity to pain modulatory structures. Twelve healthy seniors (HS) and twenty-one sAD patients experienced innocuous and moderately painful forearm pressure (1-5 kg/cm^2). Behavioral responses were recorded and scored with portions of the Pain Assessment in Advanced Dementia (PAINAD) scale. Nine of the HS and six of the sAD subjects that were tested, behaviorally, also underwent two 7-minute resting-state fMRI scans to examine functional connectivity differences for pain-related regions of interest (ROIs).
sAD subjects had greater PAINAD scores for all stimulus intensities (main effect: $F=3.89$; $p<0.05$/group*stim intensity interaction: $F=108.5$; $p<0.001$). Preliminary seed to whole-brain connectivity analysis showed reduced connectivity in sAD patients among pain modulatory ROIs (periaqueductal gray [PAG] to dorsolateral prefrontal cortex [DLPFC] & hypothalamus to DLPFC), between affective and modulatory ROIs (anterior insula to DLPFC, middle cingulate to medullary reticular formation, anterior cingulate [ACC] to PAG), and between sensory and

modulatory ROIs (mid/posterior insula to DLPFC and ACC). sAD patients also had reduced connectivity among sensory pain regions (mid/posterior insula, secondary somatosensory cortex) and between affective and sensory pain regions [$p<0.005$, whole-brain corrected, clusters>400mm^3].
The observed pattern of connectivity changes between HS and sAD suggests that while pain modulation may indeed be dysfunctional, sensory and sensori-affective integration of nociceptive information may be as well. Together, these changes may predispose sAD patients to an increase in acute pain behaviors – and an inferred increase in pain sensitivity.

Disclosures: P.A. Beach: None. **M. Miranda:** None. **K. Swanic:** None. **L.L. Symonds:** None. **D.C. Zhu:** None. **A. Bozoki:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.15/VV20

Topic: D.08. Pain

Support: Sir Henry Wellcome Postdoctoral Fellowship

Title: Functional tracing by frequency coding reveals convergence in painful and pleasant touch pathways

Authors: *L. S. LOKEN, E. DUFF, I. TRACEY;
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Abstract: We here investigated the central processing of firing rates in Aβ and CLTMs on normal and sensitized skin during ongoing pain, in order to determine how their respective firing rates influences C fibre activity to produce affective experiences spanning pain, pleasure and analgesia.
Using a within subjects design, we compared perception and central neural processing of soft brush stroking at varying velocity on normal and capsaicin sensitized skin in 19 healthy individuals while recording behaviour and functional magnetic resonance imaging (fMRI), respectively. The protocol was designed to modulate known firing rates in CLTM and Aβ afferents.
Within the area of sensitization, Aβ afferent discharge rates interact with C fibre hyperexcitability in a linear fashion such that the percept shifts from allodynia to analgesia. Outside the sensitized area, all brushing was assessed as pleasant but most efficient when producing high discharge rates in CLTM afferents. Using functional magnetic resonance imaging

(fMRI), a painful stimulus was delineated from a pleasant stimulus by activity in brain areas promoting a nocifensive response. Modeling the unique firing rate signatures of Aβ and CLTMs to fMRI data showed that the central processing of activity related to these two fibre classes were modulated in different ways depending on whether brushing was on the area of sensitization or on normal skin.
We show that brush evoked allodynia and analgesia in sensitized skin depends on Aβ fibre discharge rate and that patterns of activity from specific afferents interact centrally to produce affective sensations. Previously designated discriminative, nociceptive and hedonic circuits are not separate static channels but shared across modalities, integrated and modulated to produce affective percept.

Disclosures: L.S. Loken: None. **E. Duff:** None. **I. Tracey:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

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Program#/Poster#: 268.16/VV21

Topic: D.08. Pain

Title: Three days of MRI restraint training for imaging of awake rats causes stress-induced analgesia and decreases in exploratory behavior

Authors: *L. LOW, A. LE, L. HYSON, M. BUSHNELL;
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Abstract: Aim
Functional MRI is used in humans to study cognitive and emotional processes, including pain. In order to conduct similar studies in rodents, the animals must be awake and conscious during scanning. Since the restraint and loud noises of MRI can cause stress and alter neural responsiveness, investigators have exposed rats to 3 days of restraint and noise habituation before scanning. Nevertheless, it has not been determined whether such methods normalize pain and emotional behaviors. Here we examine the effect of 3 daily sessions of restraint and noise on pain sensitivity and emotional behavior.
Methods
12 male rats (250-300g, Charles River Laboratories) were acclimated to the animal facility for 3 days. On the 4^{th}, 5^{th} and 6^{th} days, rats were subject to either MRI restraint and loud scanner noise, or exposure to the apparatus and quiet scanner noise (6 animals in each group) for 30

minutes per day. Restrained animals were placed in a fabric 'snuggle' with head and tail protruding, and strapped into a Perspex MRI cradle, and non-restrained animals were exposed to and allowed to explore the restraint apparatus. On the 7th day, thermal sensitivity was measured with the Hargreaves apparatus, and on the 8th day, rats were tested for 5 minutes in the elevated zero maze.

Results

Restrained animals had longer thermal withdrawal latencies than non-restrained controls ($p=0.02$), consistent with stress-produced analgesia. There were no differences between groups on classic anxiety-like behavior outcome measures (time spent on/entries into open sections; $p>0.05$) but restrained animals spent less time at the boundary of the arms, suggesting a decrease in exploratory behavior ($p=0.02$).

Conclusions

These results show that MRI restraint training performed over 3 days is not sufficient to normalize pain and emotional behaviors in rats, but rather may cause stress-induced analgesia and decreases in exploration in rats. Researchers wishing to perform awake MRI imaging on rats should be aware that MRI training may produce stressed animals, potentially confounding imaging studies.

Disclosures: L. Low: None. **A. Le:** None. **L. Hyson:** None. **M. Bushnell:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

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Program#/Poster#: 268.17/VV22

Topic: D.08. Pain

Support: The Ministry of Education, Science and Technology, Republic of Korea (No. 2011 0009913 & No. 2005-0049404)

Title: Context-dependent brain responses to acupuncture needle: Pain-relieving practice or pain-evoking needling

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[2]Dept. of Brain Computer Engineering, Korea Univ., Seoul, Korea, Republic of

Abstract: Background: It is well-known that acupuncture exerts pain-relieving effect in various kinds of disease. Since acupuncture uses fine needles penetrating into specified points on the skin, it can inevitably provoke a certain degree of pain. In this aspect, people can exhibit two

different brain responses depending on the two different meanings of acupuncture needling: 'pain-relieving practice' or 'pain-evoking needling'.
Objectives: We wanted to know whether acupuncture could exert different brain responses in pain modulation of two different contexts (therapeutic practice versus painful stimulation).
Methods: Twenty-four participants were randomly divided into two groups, acupuncture treatment group (AT group, n=12) and acupuncture stimulation group (AS group, n=12). They were delivered mechanical painful stimulation using pinprick (256mN) after tactile (T+P session) and acupuncture stimulation (A+P session) in the fMRI scanner. Although two groups received identical acupuncture stimulation, the AS group was conditioned to consider acupuncture as one of painful stimulation, while the AT group as one of pain relieving modality. We compared the blood oxygen level dependent (BOLD) signal changes induced by pain after tactile (T+P session) versus pain after acupuncture (A+P session) between the two groups.
Results: When the participants considered acupuncture as an analgesic modality (AT group), acupuncture stimulation decreased enhanced BOLD signal of the mechanical painful stimulation in the insula and secondary somatosensory cortex (SII). When the participants considered acupuncture as just a painful stimulation (AS group), however, acupuncture stimulation did not affect enhanced BOLD signal to the mechanical painful stimulation in those brain areas. When we compared the BOLD signal to acupuncture stimulation itself between the two groups, acupuncture stimulation exerted greater brain activation in the nucleus accumbens (known as reward-related region) in the AT group than the AS group.
Conclusion: Under the context of therapeutic effect, acupuncture stimulation not only strengthened brain reward circuitry but also exhibits greater pain modulatory effect in the pain matrix in the brain. Our findings suggest that brain responses to acupuncture stimulation can be influenced by the context of acupuncture needle.

Disclosures: I. Lee: None. **T. Lee:** None. **H. Lee:** None. **C. Wallraven:** None. **Y. Chae:** None.

Poster

268. **Pain Imaging and Perception II**

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Topic: D.08. Pain

Support: Research Fellowship by the German Research foundation to SB

Louise and Alan Edwards Foundation’s Edwards PhD Studentship in Pain Research to WG

CIHR Operating Grant to PS

Title: Orbitofrontal cortex mediates pain modulation by monetary reward

Authors: *S. BECKER[1,2], W. GANDHI[2], F. POMARES[2], P. SCHWEINHARDT[2];
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Abstract: Rewarding stimuli have hypoalgesic effects in humans and animals. While it is known that reward and pain processing overlap in several brain regions such as the prefrontal cortex, the anterior cingulate cortex (ACC), and the insula, it remains unclear which brain regions mediate the pain modulatory effects of rewarding stimuli. Therefore, we conducted a functional magnetic resonance imaging (fMRI) study in human volunteers.
Twenty-four healthy volunteers participated in an fMRI testing session during which they played a wheel of fortune task. In this task, participants could win (reward), lose (punishment), or neither win nor lose money (neutral condition) after spinning a virtual wheel by a button press. In 64 out of 102 trials participants received brief (3 sec) mildly or moderately painful heat stimuli whilst being presented with the outcome of the wheel. Immediately afterwards, participants rated the intensity of each pain stimulus on visual analogue scales. Whole brain functional images were acquired with head coil arrays fitted for parallel imaging to minimize signal loss and image distortion in the orbitofrontal cortex using a standard echo-planar imaging sequence (3T MR-scanner, TR=2.37s, TE=25 ms, 3 mm isotropic voxel resolution) and an event-related design.
The wheel of fortune task successfully modulated participants' perception for mildly painful stimulation: stimuli were perceived as less intense after winning compared to losing money. The fMRI data showed activation of typical pain associated areas, such as left and right insula, ACC, left and right parietal operculum, and right thalamus in response to the painful stimulation. Successful pain modulation was associated with increased activity in the orbitofrontal cortex. This signal change in the orbitofrontal cortex was in turn negatively correlated with activity in the caudal ACC.
The results demonstrate that hypoalgesic effects of reward are mediated by the orbitofrontal cortex, possibly by dampening pain processing in the caudal ACC.

Disclosures: S. Becker: None. **W. Gandhi:** None. **F. Pomares:** None. **P. Schweinhardt:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.19/WW2

Topic: D.08. Pain

Support: Marie Curie IEF 273805

Title: Learning-related signals in the human spinal cord

Authors: *F. EIPPERT[1], J. C. W. BROOKS[2], Y. KONG[1], J. X. O'REILLY[1], J. FINSTERBUSCH[3], I. TRACEY[1];
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Abstract: Somatosensory afferents from the peripheral nervous system are the only direct sensory input into the dorsal horn of the spinal cord, but previous electrophysiological studies in monkeys have shown that the dorsal horn also exhibits responses to non-somatosensory (e.g. visual) stimuli, suggesting top-down signalling from the brain. To investigate the functional relevance and specificity of such responses we used functional magnetic resonance imaging (fMRI) of the human spinal cord in combination with an associative learning task, in which visual stimuli were either predictive or non-predictive of painful somatosensory stimulation.
We studied 32 healthy male subjects in an aversive learning paradigm, where visual symbols served as conditioned stimuli (CS) and painful electrical stimulation delivered to dermatome C6 served as unconditioned stimuli (US). The paradigm consisted of an acquisition phase (CS+: 66% reinforcement, CS-: no reinforcement) and an extinction phase (CS+ and CS-: no reinforcement). fMRI data of the cervical spinal cord were acquired on a 3T Siemens scanner fitted with a custom-made 22-channel spine-coil using a sequence optimized for the cervical spine; behavioural data acquisition included shock expectancy ratings as well as pupil dilation and skin conductance responses to CS and US.
The painful US resulted in significant increases in pupil dilation and skin conductance responses, as well as significant responses in the spinal cord dorsal horn. Regarding learning-related effects, we observed a significant difference between CS+ and CS- during acquisition in shock expectancy ratings, pupil dilation and skin conductance responses and all of these differences became significantly smaller during extinction. Most importantly, when we investigated responses occurring during the anticipation of painful stimulation (i.e. in the CS interval), we observed that responses in the ipsilateral dorsal horn were significantly greater for the pain-predicting CS+ than the CS- in the acquisition phase; this effect was significantly decreased during extinction.
Together, these results demonstrate that the human spinal cord shows responses to non-somatosensory stimuli, but only if these are predictive for the occurrence of painful somatosensory events.

Disclosures: F. Eippert: None. **J.C.W. Brooks:** None. **Y. Kong:** None. **J.X. O'Reilly:** None. **I. Tracey:** None. **J. Finsterbusch:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.20/WW3

Topic: D.08. Pain

Title: A study on instability of pain perception threshold

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Abstract: There are two main types of nerve fiber that detect pain: A-delta and C. C fibers also function for the perception of touch. We examined the threshold levels for pressure stimulation perceived as pain when increasing pressure stimulation is added near the ulnar nerve of small finger. We report the data on varying threshold levels for pain perception which we gained by attaching Microcones on the course of the ulnar nerve during our examination.
We prepared two types of tools. One is a stick and another is disks. The stick is 10cm long, plastic, and to generate pressure stimulation. It includes a spring inside to adjust its pressure stimulation. We made disks with and without Microtones. The disk with Microcones is 3μm tick and 1cm in diameter. The disk has 376 Microcones, each of which is 150μm tall and 20μm in diameter. The disk without Microcones is 3μm tick and 1cm in diameter. We used the disk with Microcones for pressure stimulation and another without Microcones as a placebo. The experiment participants were healthy adults. They were seated on chairs with eye masks on. Experiments started after 5 minutes of resting. The experimenter stimulated the nail fold areas on the participants' small finger. The ulnar nerve runs in nail fold. The experimenter immediately recorded the scale values on the stick when the participants report of pain. The above experiments were repeated three times per participant and the experimenter calculated the mean values. Next, experiments were carried out with the disk with Microcones attached between the Metacarpophalangeal and the Carpometacarpal joints on the dorsal of hand. For comparison, experiments were also conducted with the placebo attached on the same regions. Based on the above experiments, we compared the threshold levels for pain perception by pressure stimulation with and without Microcones.
The pain perception threshold levels did not vary when nothing was attached and a placebo was attached. Compared to these levels, the threshold levels were significantly high when the disk

with Microcones was attached. It became clear a disk without Microcones functions as a placebo. The threshold levels for pain perception were indicated not to be fixed but to vary depending on conditions. This study is considered to provide an important suggestion for reduction of pain.

Disclosures: S. Fukasawa: A. Employment/Salary (full or part-time):; TOYORESIN corporation. **S. Ide:** A. Employment/Salary (full or part-time):; TOYORESIN corporation. **H. Eda:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.21/WW4

Topic: D.08. Pain

Support: CIHR

Title: Mind wandering during painful stimulation engages the default mode and antinociceptive networks

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Abstract: Introduction: Human minds frequently wander toward thoughts unrelated to the present sensory environment, sometimes even in the face of salient events such as pain. We developed a new approach to probe brain fluctuations when people attend to pain versus when they attend to stimulus-independent thoughts during painful stimulation.
Methods: 51 healthy subjects participated in two sessions. In session one, participants performed two tasks that included epochs of painful transcutaneous electrical nerve stimulation (TENS). In an experience sampling task, participants reported whether they were attending to pain or to stimulus-independent thoughts during TENS trials. We also assessed the degree to which reaction times (RT) changed due to concurrent pain during a cognitive interference task. In session two, participants repeated the experience sampling task during fMRI and underwent diffusion-weighted imaging (DWI). Group-level fMRI contrasts were thresholded at cluster-based $Z > 2.3$ and FWE-corrected $p < 0.05$.
Results: Based on the proportions of trials in the experience sampling task with reports of

attention to pain versus attention to something else, we defined an "intrinsic attention to pain" (IAP) score. Inter-individual differences in IAP were correlated with the disruptive effect of pain on RT in the cognitive interference task ($r = 0.42$, $p = 0.003$). Attentional fluctuations away from pain occurred during decreased salience network activation, as well as increased default mode network (DMN) activation that was positively correlated with inter-individual differences in mind wandering during TENS ($\rho = 0.45$, $p = 0.011$). Psychophysiological interaction analysis revealed enhanced functional connectivity between the periaqueductal gray area (PAG), a key node of the antinociceptive pathway, and areas of the DMN (e.g. medial prefrontal cortex (mPFC), posterior cingulate cortex) during attentional fluctuations away from pain. DWI analysis revealed that inter-individual differences in PAG-mPFC structural connectivity strength (i.e., fractional anisotropy) negatively correlated with IAP ($r = -0.36$, $p = 0.009$).
Conclusion: These data suggest that everyday mind wandering may reduce pain through engagement of the default mode network and descending antinociceptive system.

Disclosures: A. Kucyi: None. **T.V. Salomons:** None. **K.D. Davis:** None.

Poster

268. **Pain Imaging and Perception II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 268.22/WW5

Topic: D.08. Pain

Title: Identification of imaging biomarkers of pain using fMRI in awake Trpv1 knock-out and wild-type rats

Authors: *J. R. YEE[1], W. KENKEL[1], K. GAMBER[2], P. SIMMONS[2], M. NEDELMAN[3], P. KULKARNI[1], C. F. FERRIS[1];
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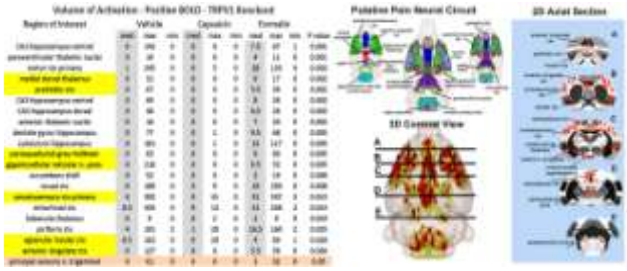
Abstract: In the present study, we used functional MRI in awake rats to follow the pain response that accompanies introduction of intradermal capsaicin into the hindpaw. To this end, we used BOLD imaging together with a 3D segmented, annotated rat atlas and computational analysis to identify the integrated neural circuits involved in capsaicin-induced pain. The specificity of the pain response to capsaicin was tested in a transgenic knock-out of the Trpv1 receptor (SAGE Labs, St Louis MO). This model contains a biallelic deletion of the Trpv1 gene, encoding for the transient receptor potential cation channel subfamily V member 1. Capsaicin, the component in chili peppers responsible for its spicy quality, is an exogenous ligand for Trpv1. Shown in the

figure to the right is a 3D reconstruction of the putative pain neural circuit. The different brain areas are coalesced into a single volume (yellow) showing the average significant BOLD signal change (red) from nine animals. The location of the brain activation can also be seen on the 2D axial sections taken from the brain atlas. As expected, capsaicin did not elicit the activation pattern in the Trpv1 knock-out rats observed in controls as shown in the table to the left. However, the intradermal injection of 3% formalin elicited a significant activation of the putative pain pathway (areas denoted in yellow). Taken together, these results provide neuroimaging evidence that the Trpv1 KO rat fails to respond to natural ligands for the Trpv1 receptor, and thus support the Trpv1-KO rat as a useful animal model for detailed investigations of neural circuitry involved in pain. In addition, these results also support the use of functional imaging in the awake rat as an effective means of generating neural activation biomarkers for use in novel analgesic discovery that can provide translational information to complement existing behavioral assays.



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Poster

268. **Pain Imaging and Perception II**

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Program#/Poster#: 268.23/WW6

Topic: D.08. Pain

Support: National Institute for Information and Communications Technology, Japan

JSPS KAKENHI Grant Number 12345678

Title: Perceptual learning of cutaneous thermal sensation

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Abstract: Human sensation can be divided into exteroception - the ability to sense one's external environment, and interoception - the ability to sense the physiological condition of our internal bodily environment. Interoception incorporates the sensory modalities of temperature and pain, the afferent pathways of which are anatomically and physiologically distinct from exteroceptive somato-sensation, being peripherally transmitted by small myelinated and unmyelinated fibres and to the brain via the spinothalamic tract. The central (cortical) representation of temperature and pain is controversial, and one major missing component of previous studies is the lack of any demonstration of perceptual learning - the hallmark of cortical processing. Here, we present preliminary data from a temperature discrimination task, in which healthy participants were required to identify very small changes in warmth and cooling of a temperature thermode attached to the leg. We used a 1-interval detection paradigm in which subjects were required to detect the presence or absence of very small pulses of temperature: either decreases from a cool baseline (25C, which selectively activates cold fibers) or increases from a warm baseline (39C, which selectively activates warm fibers), at a range of difficulties on the right or left leg. We then extensively trained subjects on one specific condition: either warmth or cooling, on either right or left, over 1 week. Subjects were then re-tested to assess whether their detection ability improved as a function of training. We showed a progressive improvement in discriminative accuracy (d prime) over the training period. Furthermore, we showed that when testing discrimination performance after training, the improvement was specific to side (left or right) and temperature (warming or cooling) of training. This provides evidence for an anatomically and functionally specific mechanism of thermosensory perceptual learning.

Disclosures: H. Mano: None. **W. Yoshida:** None. **K. Shibata:** None. **S. Zhang:** None. **M. Kawato:** None. **B. Seymour:** None. **M. Koltzenburg:** None. **M. Lengyel:** None.

Poster

269. **Tactile Sensation and Plasticity in Humans**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 269.01/WW7

Topic: D.09. Tactile/Somatosensory

Title: Superior tactile sensibility in sports involving ball handling

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Abstract: The hands have a great tactile sensitivity (Johansson et al., 1979). In tool-using sports, such as baseball and basketball, tactile sensation is crucial for successful performances. Moreover, the finger tactile sensitivity becomes finer as a result of training ("tactile learning"; Harris et al., 2001). We hypothesized that athletes in sports with ball-hand contact had greater tactile sensitivity than others. To test this hypothesis, we measured the ability to discriminate two stimuli applied concurrently to the thumb and index or ring finger. Subjects were active baseball players and active basketball players (BPs), and non-ball-hand contact sports players who had no history of sporting activity involving ball hand contact (NPs). Task was to answer the digit which was stimulated with greater number of pins. During the measurement, the first joint of each digit being tested were at rest on a box with flat surface and width of 19 mm. The each box stores two rows of four 1.3 mm-diameter pins which can be manually moved to above and below the surface. In each pair of digits, 4 sets of 32 trials (first five trials served as practice trials) with or without disturbance stimuli, which stimulate the digits 8 pins, were conducted. In each box, one of three patterns of stimulations (two, four, or six pins) was randomly provided. For the thumb and index finger pair, the rate of correct answer in the NPs significantly decreased in the presence of a disturbance ($p<0.01$), while that in the BPs did not change. For the thumb and ring finger pair, the accuracy in the baseball players and NPs significantly decreased in the presence of disturbance, but it did not change in the basketball players. The different results found in between baseball and basketball players may be derived from the difference in the way of using fingers in each sport. Baseball players mostly use index finger and middle finger when they throw a ball. On the other hand, basketball players mostly use all digits when they catch and throw a ball. It might be necessary for ball-handling athletes to have great tactile sensitivity in order to achieve successful performance. These results suggested that tactile sensitivity of BPs can be improved through sports training, and that the improvement would appear only to those fingers used in each sport.

Disclosures: M. Maetomo: None. **Y. Uchida:** None. **K. Kanosue:** None.

Poster

269. **Tactile Sensation and Plasticity in Humans**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 269.02/WW8

Topic: D.09. Tactile/Somatosensory

Support: DFG, VO 1432/7-1; SPP 1184

DFG, GO 802-7-1

Title: Age-related decline in tactile perception is delayed by dexterous use of hands at work - an ERP study

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Abstract: Age-related decline in tactile perception is evident already in middle-aged adults. However, extensive dexterous use of the fingers leads to specific expertise in tactile perception and changes in brain activation [1, 2]. The current study investigated whether also extensive and precise use of the fingers at work affects tactile perception and whether this might serve as a buffer against age-related decline.
Based on their occupation, 47 younger (38-48 years) and older (55-66 years) middle-aged workers were divided into experts (precisions mechanics) and non-experts (service employees). Participants performed tactile pattern (PDT) and frequency (FDT) discrimination tasks while their EEG was recorded. Discrimination performance and ERP components P50, N70 and P300 were analyzed.
2 (Ages) x 2 (Expertise) ANOVA revealed that in both tasks, experts outperformed non-experts (PDT: $F(1,43) = 5.42$, $p = .025$; FDT: $F(1,42) = 6.03$, $p = .018$). Age (PDT: $F(1,32) = 4.33$, $p = .045$,; FDT: $F(1,31) = 6.41$, $p = .017$) and expertise (PDT: $F(1,32) = 4.23$, $p = .048$; FDT: $F(1,31) = 4.31$, $p = .046$) led to increased N70 amplitudes. In PDT, age ($F(1,28) = 5.07$, $p = .032$) and expertise ($F(1,28) = 5.23$, $p = .030$) resulted in reduced P300 amplitudes; while in FDT an Age x Electrode interaction was revealed ($F(2,70) = 3.96$, $p = .034$) with more equally distributed P300 amplitudes in older adults.
We conclude that extensive and dexterous manual work leads to acquisition of tactile expertise that might postpone age-related decline. Comparable neurophysiological changes induced by age and expertise, presumably have different underlying mechanisms. Enlarged somatosensory N70 amplitudes might result from reduced inhibition in older adults but from enhanced, specific excitability of the somatosensory cortex in experts [3, 4]. Regarding P300, smaller amplitudes might indicate fewer available resources in older adults and, by contrast, reduced need to engage as much cognitive effort to the task in experts [5, 6].
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Disclosures: E. Reuter: None. **C. Voelcker-Rehage:** None. **S. Vieluf:** None. **A.H. Winnecke:** None. **B. Godde:** None.

Poster

269. **Tactile Sensation and Plasticity in Humans**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 269.03/WW9

Topic: D.09. Tactile/Somatosensory

Title: The Tool Ownership Illusion: Motor experience facilitates incorporation of a tool

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Abstract: Almost 15 years ago, Botvinik and Cohen discovered that when subjects watched their own hand being brushed synchronously with a fake hand, they felt like the fake hand was their real hand. This illusion, called the Rubber Hand Illusion (RHI), has since been studied by many researchers who found that a key aspect is the visual similarity between the fake hand and the subjects' hand. Indeed, the RHI arises only in presence of a fake hand and not other objects, as for example wooden blocks (even when they are shaped as a human hand). Here we tested whether functional similarity (instead of anatomical similarity) is sufficient for the illusion of ownership to extend to non-hand-shaped tools. In particular, we wanted to test whether it is possible to induce the illusion by stroking a grabber that shares the same functionality of a human hand (to grasp), despite its different visual appearance. We hypothesized that motor experience with the tool would be necessary to induce the illusion.
We tested subjects in a modified version of the classical RHI paradigm. Subjects were asked to observe a grabber being stroked synchronously (test condition) or asynchronously (control condition) with their own (hidden) right hand. After this, they were asked to localize their own

right hand and to answer a questionnaire. This procedure was applied twice, before and after a short period of tool-use consisting in grasping and lifting objects with the grabber. The amount of proprioceptive drift (toward the tool) in the localization of the index finger after stroking was calculated and treated as a measure of the amount of illusion experienced by the subjects. Crucially, subjects had no previous experience with the tool prior to the experiment.
Results from the localization task showed that synchronous stimulation induces a significant drift toward the tool, but only after having using it. . These results seem to confirm the importance of both visual and functional similarity. However, performances at the questionnaire suggest that an illusory sense of ownership over a tool can be consciously experienced even without motor experience.

Disclosures: L. Cardinali: None. **A.C. Roy:** None. **J.C. Culham:** None. **A. Farnè:** None.

Poster

269. **Tactile Sensation and Plasticity in Humans**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 269.04/WW10

Topic: D.09. Tactile/Somatosensory

Support: Max Planck Society

Title: Shifting somatosensory detection thresholds with transcranial alternating current stimulation in a phasic manner

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Abstract: Various neural oscillations are associated with brain functions such as somatosensory perception. The amplitude of the sensorimotor mu rhythm for instance, can be linked to the perception of near-detection-threshold somatosensory stimuli (Linkenkaer-Hansen et al., 2004). Furthermore the phase of neuronal oscillations affects the perception of near-threshold stimuli (Busch et al., 2009). Non-invasive brain stimulation methods like transcranial alternating current stimulation (tACS) may offer the possibility to modulate oscillatory amplitude (Zaehle et al., 2010) as well as phase (Neuling et al., 2012).
We examined the effect of tACS applied at participants' individual mu frequency on threshold levels of somatosensory perception. We hypothesized that (a) tACS modulates somatosensory perception thresholds compared to sham stimulation and (b) perception thresholds vary as a

function of the phase of tACS.
In a randomized, single-blinded, crossover design, 17 participants (mean age: 27; female: 10) underwent a combined EEG/tACS experiment in two separate sessions (real or sham tACS). We first operationalized subjects' individual mu-frequency as the maximum event related desynchronization to suprathreshold electric stimuli presented with electrodes at the right index finger. Subsequently somatosensory detection thresholds were continuously determined in a block of 16 minutes using an adaptive staircase procedure of weak electric stimuli. In between this detection task, 5 minutes of tACS was applied at the individual mu frequency in a bilateral montage over both primary somatosensory cortices (S1). For sham, 30 s of 1 mA random noise stimulation was applied.
We found no differences in the average somatosensory perception thresholds between real and sham stimulation. However, during tACS, somatosensory detection thresholds changed as a function of the phase of tACS with thresholds differing maximally for stimuli presented at opposite phases of the tACS signal curve. Compared to thresholds derived from 10,000 synthetic phase-shuffled datasets this difference was significant at a corrected p-value of $p<.005$.
We conclude that tACS applied at the individual mu frequency over S1 is capable of shifting somatosensory detection thresholds in a phase-dependent manner. Our findings suggest that functionally relevant intrinsic oscillations may be modulated using non-invasive brain stimulation.

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Poster

269. **Tactile Sensation and Plasticity in Humans**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 269.05/WW11

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant R01 EB000215

Title: Direct visualization of cns sensory network damage in the pediatric brachial plexus injury patient: an bold fmri and fcmri study under 3t

Authors: *R. LI[1], J. A. MACHOL, IV[2], N. FLUGSTAD[2], X. LIU[3], J.-G. YAN[2], H. S. MATLOUB[2], J. S. HYDE[1];
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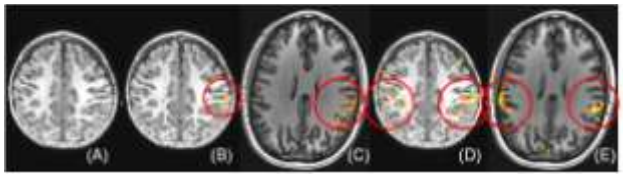
Abstract: PURPOSE: Upper trunk lesion involving the C5-C6 roots is the most common in brachial plexus birth palsy. For any nerve transfer procedure, although all the patients are operated in the same way to repair the nerve, the clinical outcome may vary. Studies have shown brain plasticity might be responsible for this inter-subject variation. With this study, we set up a reliable and non-invasive method to directly visualize the brain network damage caused by significant PNS injury using fMRI and fcMRI in a pre-operative pediatric brachial plexus injury infant.
METHODS: An 11-month-old female with left brachial plexus birth palsy was evaluated. The patient was noted to have injury to the C5-C6 nerve roots resulting in 0/5 shoulder external rotation and posterior deltoid function. This pathology was confirmed via pre- and intra-operative EMG and during post-scan surgical exploration. BOLD fMRI and fcMRI were performed prior to surgery under 3T. EPI imaging was obtained while a timed air-puff mechanism delivered somatosensory stimulus to the C5-C6 dermatome (lateral deltoid region). This was completed in duplicate for the injured side (left) and then again for the healthy side (right). The infant is asleep while the adult is awake during the scan.

RESULTS and Discussion: No sensory response can be detected when the deltoid region of the injury side was stimulated (A). A distinct fMRI sensory response can be found in the non-injury side of the birth palsy patient (B) and the healthy adult (C). Since the nerve injury was limited to C5-6 nerve root in brachial plexus nerve roots, diminished somatosensory network contralateral to the nerve injury was demonstrated while the other hemisphere remained normal (D). The same network remains symmetrical in healthy adult (E). This study can be applied to almost all the fMRI/fcMRI studies dealing with sensory function in children and adults. The subject could be awake or asleep and the result remains consistent.Ultimately, this model will be utilized to prospectively investigate cortical sensory plasticity after nerve transfer for brachial plexopathy treatment.



Disclosures: R. Li: None. **J.A. Machol:** None. **N. Flugstad:** None. **X. Liu:** None. **J. Yan:** None. **H.S. Matloub:** None. **J.S. Hyde:** None.

Poster

269. **Tactile Sensation and Plasticity in Humans**

Location: Halls B-H

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Topic: D.09. Tactile/Somatosensory

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P01-AT002048

Title: Sensory discrimination and adaptation reflect reorganization in primary somatosensory cortex in carpal tunnel syndrome

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Abstract: INTRODUCTION: Carpal tunnel syndrome (CTS), a median nerve entrapment neuropathy, is characterized by functional and structural neuroplasticity in primary somatosensory cortex (S1). As tactile discrimination and adaptation assessed with dual site conditioning stimuli has been associated with S1 activity, we aimed to investigate the relationship between tactile discrimination and somatotopic reorganization in CTS. METHODS: Subjects (46 CTS and 22 age-matched healthy controls, HC) received vibrotactile stimulation on median nerve innervated index (D2) and middle (D3) fingers using a vibrotactile stimulator (CM-4, Cortical Metrics, NC). The stimulus on one finger gradually increased while the other decreased either 0.5 or 3.0 sec after onset of stimulation (pre-conditioning stimulus). Subjects reported the finger with increasing stimulus. The discrimination response time difference between the two pre-conditioning durations was used as adaptation index, while discrimination accuracy was also evaluated. FMRI data using an event-related design were acquired (TR/TE = 2000/30ms, 32 axial slices, voxel size = 2.1x2.1x2.5mm) on a 3.0T Siemens Trio equipped with 32-channel head coil. Fingers (D2, D3) were stimulated with MR-compatible vibrotactile device using a piezoelectric transducer. Discrimination accuracy, response time and adaptation metrics were compared between groups. FMRI data were analyzed using a general

linear model. Group maps were created and corrected for multiple comparisons at $p<0.05$. Peak activations in contralateral S1 from each subject's finger stimulation were extracted and D2/D3 separation distance was compared between CTS and HC, and between good and poor discrimination subgroups (based on normative HC data) within CTS. D2/D3 distance was also correlated with the adaptation metric.
RESULTS: Discrimination accuracy was significantly lower in CTS compared to HC ($p<0.05$). Response time in the poor subgroup was significantly shorter compared to the good subgroup ($p<0.05$). Vibrotactile stimulation produced more overlapped activation in contralateral S1 for CTS compared to HC. D2/D3 separation distance in CTS was significantly lower compared to HC ($p<0.05$). Additionally, D2/D3 distance in CTS was positively correlated with adaptation ($r=0.38$, $p=0.02$), while negatively correlated with response time ($r=-0.46$, $p<0.01$). Therefore, the greater the D2/D3 distance, the greater the adaptation and faster response.
CONCLUSIONS: S1 reorganization in CTS is maladaptive, as it is associated with poor discrimination performance and diminished adaptation.

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Poster

269. **Tactile Sensation and Plasticity in Humans**

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Topic: D.09. Tactile/Somatosensory

Support: European Community's Seventh Framework Programme FP7/2007-2013 under grant agreement number PITN-GA-2008-214728

BrainGain Smart Mix Programme of the Dutch Ministry of Economic Affairs and the Dutch Ministry of Education, Culture and Science

Title: The effect of task instructions on haptic texture processing

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Abstract: The haptic perception of spatial density and roughness of a dot pattern texture are characterized by different psychometric curves, i.e. a linear and a non-linear dependence on inter-element spacing respectively. Neuroimaging literature suggests a corresponding dissociation in the underlying cortical networks. However, most imaging studies did not systematically vary roughness or density levels, or focused only on a single feature. Studies that considered both aspects concentrated on single cortical regions. A repetitive TMS (rTMS) study for example found that roughness perception was impaired by applying rTMS to the somatosensory cortex, while reports of spatial density were altered after rTMS to the occipital cortex (Merabet et al., 2004). However, a similar disruptive effect for spacing discriminations was also reported for TMS targeting the somatosensory cortex (Zangaladze et al., 1999). In the current fMRI study we try to resolve this controversy by focusing on multiple cortical areas previously reported for roughness and density perception. We investigated the effect of task instructions in these regions using a haptic roughness and density perception task with identical stimuli. We also explored whether the same cortical areas contribute to the distinction of different levels of roughness or density.
Whole brain fMRI time series were collected while subjects rated either perceived roughness or spatial density of embossed dot patterns varying in the mean center-to-center dot spacing. Three different models were considered for multivariate pattern analysis of the fMRI signal, which were based on the individual psychophysical results: (1) high vs. low roughness, (2) high vs. low density and (3) roughness vs. density irrespective of the spacing differences of the stimuli.
The roughness and density rating task activated a similar cortical network. A subset of these regions showed a contribution to task classification, namely the prefrontal cortex, the postcentral gyrus, posterior parietal areas, the operculo-insular cortex as well as early and higher-order visual regions. However, brain areas distinguishing between high and low levels of spatial density were different from regions showing differential activation patterns for high and low perceived roughness. Our results replicate and extend earlier findings by showing a contribution of anterior and posterior parietal regions as well as early occipital regions to a spatial density judgment task and a contribution of the operculo-insular cortex as well as ventral temporal regions to haptic roughness estimation.

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Poster

269. **Tactile Sensation and Plasticity in Humans**

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Topic: D.09. Tactile/Somatosensory

Support: ERC Grant Parietalaction, 323606

Neuroseeker - Large Scale Integrated Project, 600925

Title: Tracing hand localization in humans: Intracortical responses to median nerve stimulation

Authors: F. CARUANA[1], P. AVANZINI[2], R. O. ABDOLLAHI[3], I. SARTORI[4], R. MAI[4], G. LO RUSSO[4], G. RIZZOLATTI[1], *G. A. ORBAN[2];
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Abstract: It is known that there is a multiple somatosensory hand representation in the primate cortex. Most of the evidence in humans comes from the fMRI studies, which lack temporal resolution, and has a limited spatial resolution. In the present study we used the opportunity of standard median nerve stimulation test in presurgical evaluation of epileptic patients implanted with depth electrodes, to map cortical regions processing somatosensory hand information and investigate the time course of their responses. Electrical stimulation of the median nerve was performed in 30 patients, who provided their informed consent. We investigated the power in five frequency bands: alpha (8-12 Hz), beta (13-30 Hz), and three gamma bands (50-100; 100-200; 200-300 Hz) using continuous Morlet' wavelet analysis in 10ms time bins. The intracortical recording sites (n=2.200) were recovered using an in-house developed software. The responses where the gamma band power (50-100 Hz) showed a significance increase in at least one time bin (n= 261) after stimulation were analyzed. Their time course was classified with a K-mean clustering technique. Seven clusters were recovered, of which six were phasic and one tonic lasting 300ms. Three clusters with strong phasic responses were localized in SI (areas 3b, 1, 2) as well as in phAIP. Two clusters with weaker phasic responses occurred in a wide swath of cortex surrounding SI, including medial wall, the premotor cortex, anterior intraparietal sulcus and neighboring supramarginal gyrus, posterior STG, and parietal operculum. The two last clusters had very focal localizations. One cluster with a delayed phasic response occurred only in the dorsal precentral gyrus. The tonic cluster occurred predominantly in parietal operculum (OP1-3) and neighboring insula. In addition, a few such responses occurred in pMSTv and caudal inferior frontal gyrus. The time course of the median nerve response distinguishes SI from SII, the former being phasic and likely corresponding to the classic N20 potential of the SEP. In addition our results clearly show that additional cortical areas, outside S1 and SII, are involved in somatosensory processing of hand information. In particular, the involvement of phAIP and ventral premotor cortex is in agreement with monkey single cell studies. These results indicate

that intracerebral recordings may provide spatio-temporal mapping of function at levels far beyond those offered by functional imaging.

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Poster

269. **Tactile Sensation and Plasticity in Humans**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 269.09/WW15

Topic: D.09. Tactile/Somatosensory

Title: Leg somatotopy in the primary somatosensory cortex: A 7 Tesla fMRI study in humans

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Abstract: The primary somatosensory cortex (S1) is located within the postcentral gyrus, corresponding to Brodmann areas (BA) 3a, 3b, 1, and 2. S1 responds to tactile stimulation, with each region of the contralateral body being represented at a specific position in the postcentral gyrus. This organization, called somatotopy, was first described in humans by Penfield and Boldrey [1]. While fMRI has been recently used to map representations of individual finger, to present there are no studies mapping the cortical representations of the entire leg (from the hip to the toes) in individual subjects. We here extended previous 7T fMRI work [2] to non-invasively map 6 distinct parts of the leg in human S1.
Fourteen right-handed subjects were scanned on a 7T scanner. Functional images comprised 28 axial slices (1.3 mm3 isotropic voxel size) placed over the postcentral gyrus. Tactile stimulation was delivered on 6 regions (hip, thigh, calf, sole, big toe, and small toe) of the right and left leg by manual stroking of each body part for 20s, followed by 10s of rest. Preprocessing of fMRI data included slice timing correction, spatial realignment, and smoothing (FWHM=2mm). A GLM analysis was carried out to identify the cortical representation of the stimulated body parts within BAs 3b, 1, and 2. Finally, a two-point discrimination task was performed to measure tactile acuity for each region.
Within each individual subject, we observed a somatotopic organization of the leg representations, located in the superior and medial portion of the postcentral gyrus. Leg representations showed a lateral-to-medial organization with the hip localized in a more lateral position, and the small toe localized more medially. Thigh, calf, sole, and big toe were localized

in intermediate positions. The cortical volumes did not correlate with tactile acuity. The analysis of the BOLD response showed greater cross-region responsiveness in BA 2 compared to BAs 3b and 1.
The observed somatotopic organization of S1 is consistent with the literature. The method developed allows mapping the leg representations in S1 in individual subjects. These results indicate that ultra-high field fMRI offers the possibility to non-invasively study the cortical representations not only of the fingers but also of other body parts, such as the legs.
Reference
[1] Penfield et al., Brain, 1937
[2] Martuzzi et al., Hum Brain Mapp, in press

Disclosures: M. Akselrod: None. **R. Martuzzi:** None. **W. Van der Zwaag:** None. **J. Sulzer:** None. **R. Gassert:** None. **O. Blanke:** None.

Poster

269. **Tactile Sensation and Plasticity in Humans**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 269.10/WW16

Topic: D.09. Tactile/Somatosensory

Support: NWO Rubicon 446-10-015

NWO Veni 451-12-009

Title: Tactile localization during self-generated and passive arm movements

Authors: *F. MAIJ[1,2], A. M. WING[2], P. MEDENDORP[1];
[1]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands;
[2]Univ. of Birmingham, Birmingham, United Kingdom

Abstract: It has been shown that people make systematic errors when localizing a brief tactile stimulus that is delivered to the index finger near the time of an arm movement. These errors are likely the result of how the brain integrates the tactile input and information about arm position. The latter information can be based on afferent proprioceptive feedback or on a predictive efferent motor command, or a combination of both. Here we tested the role of predictive motor commands in tactile localization by comparing localization errors during self-generated versus passive arm movements. Subjects (n=6) were instructed to localize a tactile stimulus that was presented to their index finger near the time of actively generated fast-targeted arm movements (100 trials, 35 cm displacement). Each self-generated movement was passively replayed using a

robotic assistance (Phantom) in randomized order. We documented the EMG activity to ensure no muscular activity during the passive motion condition. We found no differences in the localization error patterns between self-generated and passive arm movements. This is consistent with the notion that proprioceptive feedback, rather than a predictive motor command, plays an important role for tactile localization. To further test the role of dynamic motor feedback, we tested how the localization errors depended on the skewness of the movement velocity profile, keeping arm displacement constant. This revealed a significant relationship, suggesting that subjects use the instantaneous movement trajectory, not overall displacement, in tactile localization. We present an optimal integration model to explain these findings.

Disclosures: F. Maij: None. **A.M. Wing:** None. **P. Medendorp:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 270.01/WW17

Topic: D.15. Basal Ganglia

Support: NIH Grant RO1NS031768

NINDS Center Grant P30NS045892

Title: ERK/MAPK signaling during striatonigral (direct) pathway development is required for normal motor control

Authors: *S. R. HUTTON, Y. WU, W. D. SNIDER;
UNC Neurosci. Ctr., Univ. North Carolina, CHAPEL HILL, NC

Abstract: In response to dopaminergic and glutamatergic signaling, the ERK/MAPK intracellular signaling pathway is hypothesized to regulate striatal medium spiny neuron (MSN) activity and motor behavior via modulation of synaptic plasticity and gene transcription. Consistent with this idea, global disruption of ERK/MAPK activity within the striatum has been documented in a number of neurological disorders and an increase in ERK/MAPK activity is linked to the development of L-DOPA-induced dyskinesias in Parkinson's patients. However, pharmacological studies assessing ERK/MAPK function in striatal circuits have been unable to ascertain the cell-specific contribution of ERK/MAPK signaling in direct versus indirect pathway MSNs. Here, we have delineated functions of ERK/MAPK signaling by conditionally deleting MEK1/2 ($Mek1^{Floxed}$,$Mek2^{KO}$) and ERK1/2 ($Erk1^{KO}$,$ERK2^{Floxed}$) using Drd1a-Cre and Drd2-Cre lines to target direct and indirect pathway MSNs. We report that conditional deletion

of MEK1/2 or ERK1/2 in direct pathway MSNs results in striking motor deficits including phenotypes associated with Parkinson's disease such as the induction of severe hypokinesia in a novel environment. Analysis of direct pathway projections to subthalamic targets revealed no overt disruption, suggesting that these phenotypes result from disruptions in neuronal function. Interestingly, mice in which constitutively active ERK/MAPK signaling is conditionally expressed in direct MSNs (caMEK1 mouse line) show increased motor ability including hyperkinesia, greater motor learning, and increased climbing behavior. Surprisingly, when targeting the indirect pathway MSNs, both the deleted- and gain-of-function ERK/MAPK mouse lines show mild changes in motor behavior. Our results suggest that ERK/MAPK signaling has critical and specific functions in the development of direct pathway-regulated motor behaviors.

Disclosures: S.R. Hutton: None. **Y. Wu:** None. **W.D. Snider:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 270.02/WW18

Topic: D.15. Basal Ganglia

Support: The Human Frontiers Science Program RGP0036/2009 - C

Precursory Research for Embryonic Science and Technology

Research on Priority Areas 23115718

Title: Representation of the aversive information in the primate caudate

Authors: *Y. UEDA, K. TOKITA, K. NAKAMURA;
Dept. of Physiol., Kansai Med. Univ., Osaka, Japan

Abstract: The prediction and appreciation of rewards influence organisms' motor control, decision making, and learning. It has been thought that the basal ganglia, especially the striatum, their input channel, are involved in the process. The brain is also equipped with neural circuits to utilize aversive information for decision making. However, little is known about whether and how aversive information is processed for motor control, decision making, and learning in the striatum.
To answer the question, we recorded single neuronal activity in the caudate nucleus while a monkey (*Macaca fascicularis*) performed a choice saccade task to acquire a reward and avoid a punishment. In the task, three fractal images were separately associated with rewarding a drop of

juice, a neutral tone, or an aversive airpuff. After fixation on the central fixation point for 1 sec, two out of the three images were presented as a pair (i.e. reward vs. tone, reward vs. airpuff, or tone vs. airpuff) simultaneously in left and right side of the fixation point. The animal was then required to choose one of two images by making saccadic eye movements. Behavioral analysis revealed that the animal learned to choose a reward-associated image and to avoid an airpuff-associated image.
We found that caudate neurons' activity was indeed influenced by the expectation or receipt of the aversive outcomes. Specifically, out of 74 task-related neurons, 32 (43%) neurons showed post-cue, peri-saccadic, or post-outcome activity whose magnitude was differently modulated depending on another counterpart of the pair even for the same direction of saccades to obtain the same rewards (i.e. the chosen image was associated with rewards but activity was different depending on the unchosen image associated with tone or airpuff). Another group of neurons exhibited an enhancement in post-cue or in post-outcome activity when the aversive image was avoided regardless of the outcomes (i.e. the chosen of image was associated with reward or tone and avoided image was associated with airpuff). These results suggest that the aversive information, integrated with the reward information, is processed in the striatum that would be a neuronal substrate of value-based decision making.

Disclosures: Y. Ueda: None. **K. Tokita:** None. **K. Nakamura:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

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Program#/Poster#: 270.03/WW19

Topic: D.15. Basal Ganglia

Support: AA015164

Samuel C. Johnson Genomics of Addiction Program

Title: Neurotensin receptor type 1 in the nucleus accumbens regulates locomotor activity

Authors: *C. ADAMS, D. J. HINTON, A. OLIVEROS, D.-S. CHOI;
Mayo Clin., Rochester, MN

Abstract:
Activation of either dopamine D1 or D2 postsynaptic receptors in the nucleus accumbens (NAc) generally evokes locomotor activity. Interestingly, neurotensin receptor type 1 (NTS1) is highly expressed in the striatum and on midbrain dopaminergic neurons, which overlaps with the

expression of D1 and D2 receptors. Not surprisingly, neurotensin is known to modulate dopaminergic neurotransmission in the NAc. Systemically administered neurotensin receptor agonists and overexpression of NTS1 in the NAc has been demonstrated to inhibit psychostimulant-mediated hyperlocomotion. However, it remains unknown whether striatal NTS1 signaling suppresses D1 and D2 receptor-mediated hyperlocomotion. Therefore, our goal was to determine the effects NTS1 activation in the NAc on basal activity and dopamine receptor-mediated hyperlocomotion. PD149163, a selective NTS1 agonist that readily crosses the blood brain barrier, was used to examine the locomotor effects of NTS1 stimulation. Systemically administered PD149163 dose-dependently reduced locomotor activity of C57BL/6J mice in an open-field experiment. Moreover, mice did not develop tolerance when they were given 3 repeated injections. Microinjection of PD149163 into the NAc reduced basal locomotor activity whereas locomotor activity was not altered when PD149163 was microinjected to the medial prefrontal cortex. Our data indicate that NTS1 activation in the NAc reduces locomotor activity and this may be through modulation of dopaminergic signaling in the NAc.

Disclosures: C. Adams: None. **D.J. Hinton:** None. **A. Oliveros:** None. **D. Choi:** None.

Poster

270. **Basal Ganglia: Physiology**

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Support: NIH Grant DA05312 (GVR)

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NSF Grant 0904912 (JMB)

NSFGrant GRFP (AN)

Title: Discerning linear and nonlinear dynamics in the corticostriatal motor system with Kernel Granger causality

Authors: *A. MURPHY-NAKHNIKIAN[1], G. V. REBEC[2], L. L. DWIEL[2], L. M. GRASSE[2], Z. P. TRITSCH[2], J. M. BEGGS[2];
[1]Program Neurosci., Indiana Univ., BLOOMINGTON, IN; [2]Program in Neurosci., Indiana Univ., Bloomington, IN

Abstract: Our group demonstrated that corticostriatal influence is bidirectional, with dorsal striatum (dStr) exerting reciprocal control of primary motor cortex (M1) during spontaneous motor output (Nakhnikian et al., 2013, J. Neurophy, In Revisions). Interestingly, we found that dStr influence over M1 vanishes almost entirely during anesthesia and sleep, and increases with motor output. Here, we applied Granger causality (GC), a temporal-precedence causality metric based on the general linear model. A major limitation of conventional GC is that it is insensitive to nonlinear interactions. To overcome this problem, Marinazzo et al (2008, PRL, 100;14) propose a method for mapping nonlinear features of a data set to a linear space using the theory of Reproducing Kernel Hilbert Spaces. In the present study, we used kernel GC to analyze local field potentials (LFPs) isolated in M1 and dStr of rats: 1) under anesthesia, 2) sleeping, and 3) engaging in a variety of spontaneous behaviors. We used the inhomogeneous polynomial kernel, a kernel that has well-known properties (Shawne-Taylor and Cristianini, 2004, Cambridge University Press), and is appropriate for GC analysis (Ancora and Stramaglia, 2006, Phys Rev. E 70). When the kernel order is one, corresponding to the linear case, we find the same causality patterns revealed by conventional GC analysis, lending credence to Marinazzo et al.'s method. As the kernel order increases, we find increasing GC during some spontaneous behaviors, but not during anesthesia and sleep. Furthermore, we find that while M1->dStr drive exceeds dStr->M1 drive in the linear case, in some cases dStr->M1 drive exceeds M1->dStr drive at higher model orders. This is a particularly interesting finding given the anatomy of the basal ganglia. Striatal information must traverse a polysynaptic loop to influence cortical targets, and nonlinear interactions between the time-series could reflect increased complexity in the temporal precedence patterns. Drive in the M1->dStr, though lower than dStr->M1 drive at higher model orders, is nevertheless higher than the M1->dStr connection observed in the linear case. Our findings thus suggest that information flow in both the corticostriatal and striatocortical directions exhibits nonlinear patterns that must be considered when analyzing this system.

Disclosures: A. Murphy-Nakhnikian: None. **G.V. Rebec:** None. **L.L. Dwiel:** None. **L.M. Grasse:** None. **Z.P. Tritsch:** None. **J.M. Beggs:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

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Program#/Poster#: 270.05/WW21

Topic: D.15. Basal Ganglia

Support: NIMH Grant MH078824

NIMH Grant MH55987

CIHR

Grass Foundation

Title: Naturalistic burst stimulation drives opposing patterns of heterosynaptic plasticity at two inputs to a songbird motor cortex analogue

Authors: *W. H. MEHAFFEY[1], A. J. DOUPE[2];
[1]UCSF, Ctr. for Integrative Neurosci., San Francisco, CA; [2]Psychiatry, UCSF, San Francisco, CA

Abstract: The avian song system has been a powerful model system to study the neural basis of learned complex motor skills, and in particular, learned vocalizations. The zebra finch (Taeniopygia guttata) sings a precise song consisting of a number of distinct elements ('syllables'), organized sequentially into repeating motifs. A well-defined set of specialized brain nuclei underlie this vocal-motor behavior, including motor cortical analogues (HVC, and RA, the Robust Nucleus of the Archistriatum), and a 'cortico'-basal ganglia loop (the Anterior Forebrain Pathway, the AFP) homologous to cortico-basal ganglia loops of mammals. Specifically, HVC inputs to RA are required to produce learned components of song, while AFP inputs are required for song variability and plasticity. HVC and LMAN inputs converge onto single RA neurons, suggesting that RA is an important site for plasticity that could shape song. Despite a wealth of knowledge about the roles of specific nuclei in song production and plasticity, a mechanistic understanding of how song changes are reflected in synaptic and neuronal dynamics remains unclear.

We show here, in 60-120 d old (adolescent-adult) zebra finches, that we can induce synaptic plasticity at the inputs to RA from its two major input pathways, LMAN and HVC. Naturalistic stimulation protocols based on patterns of high frequency spikes observed in in vivo extracellular recordings proved important in inducing changes in the synaptic strength of both input pathways. These changes in synaptic strength depended on firing in both pathways, and in particular required bursting of the LMAN inputs like that seen in LMAN when birds sing alone ('undirected' song). Strikingly, activity in LMAN and HVC inputs caused opposing shifts in the strengths of the two input pathways, such that when one was potentiated, the other was depressed. Moreover, when the relative timing between HVC and LMAN stimulation times was shifted, both the magnitude and the valence of the changes in synaptic strength changed, although the shifts in strength remained opposite for the two inputs.

Together, these data suggest that synaptic plasticity in avian motor cortical circuitry requires patterns of stimulation known to be present during song production. The opposing changes in synaptic strength in the two input pathways could underlie the dynamic shift from LMAN-driven variability to HVC-driven song stabilization that is thought to occur during song sensorimotor learning. Furthermore, the dependence of these changes on the relative timing of input stimulation raises the possibility that this timing carries information important to the learning process.

Disclosures: W.H. Mehaffey: None. **A.J. Doupe:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 270.06/WW22

Topic: D.15. Basal Ganglia

Title: Intrinsic feedback pathways shape the basal ganglia output and modulate voluntary movement

Authors: *J. BROWN, J. T. DUDMAN;
Janelia Farm Res. Campus, HHMI, Ashburn, VA

Abstract: Dysfunction of the basal ganglia (BG) produces severe deficits in the timing, initiation, and vigor of voluntary movements. These diverse impairments suggest a control system gone awry. In engineered systems feedback is critical for stable control. By contrast, functional models of the BG focus on intrinsic feed-forward circuitry. The substantia nigra pars reticulata (SNr) is the main output nucleus of the dorsal BG in rodents. SNr neurons project to premotor areas outside of the BG, but also elaborate axonal collaterals within the SN. These intranigral collaterals are the sole source of direct feedback on the afferent output of the BG. We have explored the role of local inhibitory feedback within the SNr in awake, behaving mice. Using a transgenic mouse line and targeted virus injection we expressed channelrhodopsin-2 specifically in SNr projection neurons. SNr unit activity was recorded using extracellular recording arrays while a small population of SNr projection neurons were optically stimulated. Robust light evoked activity could be recorded from units close to the light source, while robust inhibition was observed from units surrounding the excited neurons. The transient feedback inhibition was eliminated following blockade of local inhibition. Kinematic analysis of movement revealed diverse roles of intranigral feedback inhibition. These results suggest that feedback inhibition mediated by axonal collaterals of SNr projection neurons are critical determinants of both the output of the BG and voluntary movement. Thus, despite its absence from canonical models, our results suggest intrinsic feedback inhibition could be an essential feature of BG function.

Disclosures: J. Brown: None. **J.T. Dudman:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

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Program#/Poster#: 270.07/XX1

Topic: D.15. Basal Ganglia

Support: Ellison Foundation AG-NS-0944-12

Salk Innovation Research Fund

Title: Functional dissection of the cortical control of direct versus indirect pathways in the striatum

Authors: *J. R. KLUG[1], F. OSAKADA[2], E. M. CALLAWAY[2], X. JIN[1];
[1]Mol. Neurobio. Lab., [2]Systems Neurobio. Lab., Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: The dorsal region of the striatum and their cortical inputs are involved in action learning and selection. These abilities are compromised in human patients with neurological disorders affecting the striatum and its afferents, like Parkinson's and Huntington's disease. Medium spiny neurons (MSNs), the principal GABAergic projection neurons of the striatum, receive dense glutamatergic inputs from various cortical regions as well as thalamic nuclei. There are two intermixed subpopulations of MSNs: dopamine receptor 1 - containing (D1-MSNs, direct pathway) and dopamine receptor 2 - containing (D2-MSNs indirect pathway). While the two major neural pathways constitute the core components of the classic model of basal ganglia, very little is known about the connectivity and specificity of their cortical inputs. For example, the functionality and plasticity of excitatory synapses onto D1- vs. D2-MSNs from different cortical subregions remains unknown. In order to address these fundamental questions, we have used AAV-Cre-dependent helper viruses along with a modified rabies virus system (SAD delta G Rabies) to selectively visualize and manipulate cortical neurons projecting to D1- or D2-MSNs, respectively. Helper viruses are injected into subregions of dorsal striatum of D1-Cre or A2a-Cre mice, followed by an injection of modified rabies virus in the same location to allow retrograde monosynaptic tracing and expression of Channelrhodopsin-2 (ChR2-mCherry) or Archaerhodopsin (Arch-GFP) in presynaptic cortical neurons. The region and layer distributions of labeled cortical neurons are found to be consistent with reported anatomy. The functional expression of ChR2 or Arch was verified by electrophysiological recording both in brain slice and in vivo. An optical system designed for delivering fine-scale spatiotemporal light patterns was used to functionally map corticostriatal synapses. Optic fiber based optogenetic stimulation was employed to examine the contribution of ChR2-expressed corticostriatal neurons in different cortical subregions to operant learning. This system provided a unique opportunity to dissect different excitatory inputs to D1- vs. D2-MSNs and importantly, the contribution of region- and pathway-specific corticostriatal subcircuits to behavior.

Disclosures: J.R. Klug: None. **F. Osakada:** None. **E.M. Callaway:** None. **X. Jin:** None.

Poster

270. **Basal Ganglia: Physiology**

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Program#/Poster#: 270.08/XX2

Topic: D.15. Basal Ganglia

Support: JPN Grant Sci. Res.-B 2529001

JPN Grant Challeng Expl. Res. 23650202

Title: Modulation of the excitability of hindlimb motoneurons by the basal ganglia efferents to the brainstem in relation to the control of postural muscle tone and locomotion in the decerebrate cat

Authors: *K. TAKAKUSAKI[1], T. NOZU[2], T. OKUMURA[3];
[1]Reserach Ctr. for Brain Function and Med. Engin., [2]Dept. of Regional Med. and Educ., [3]Dept. of Gen. Med., Asahikawa Med. Univ., Asahikawa/Hokkaido, Japan

Abstract: Locomotion requires appropriate level of postural muscle tone. Gait disturbance is one of the cardinal symptoms in patients with Parkinson's disease (PD). Efferents from the basal ganglia project to the brainstem in addition to the cerebral cortex via the thalamocortical loops. Basic neuronal systems responsible for controlling postural muscle tone and locomotion are located in the brainstem and spinal
cord. Because the output from the basal ganglia is considered to be increased in PD, the present study was designed to understand how the basal ganglia efferents to the brainstem contribute to the control of postural muscle tone and locomotion. For this purpose, changes in postural muscle tone and intracellular activities of hindlimb motoneurons were examined in acutely decerebrated cat preparations (n=14) where basal ganglia structures were mostly removed except for the substantia nigra. Repetitive electrical stimulation (50 Hz, 20-40μA) applied to the midbrain locomotor region (MLR) generated rhythmic membrane oscillations in motoneurons, so-called fictive locomotion. The same type of stimulation applied to the ventrolateral part of the pedunculopontine tegmental nucleus (PPN) resulted in muscular atonia that was associated with membrane hyperpolarization of motoneurons. On the other hand, stimulation of the locus coeruleus (LC) increased the level of muscle tone and depolarized membrane potentials of extensor and flexor motoneurons. Although conditioning stimulation (100Hz, 40-60 μA) applied to the substantia nigra pars reticulata (SNr), a basal ganglia output nucleus, did not alter the excitability of motoneurons, it attenuated the rhythmic membrane oscillations and generated

tonic firing of both extensor and flexor motoneurons. Particularly, amplitude of hyperpolarizing phases of membrane oscillations was reduced. In addition, conditioning SNr stimulation greatly reduced the PPN-induced membrane hyperpolarization of extensor and flexor motoneurons. However the SNr stimulation did not reduced the LC-induced membrane depolarization, it rather facilitated the depolarization. Microinjections of bicuculline, a $GABA_A$-receptor antagonist, into the PPN/MLR areas blocked the effects by stimulating the MLR/PPN. These results suggest that an increase in the GABAergic basal ganglia output from the SNr to the MLR/PPN area terminate locomotion by decreasing the activity of central pattern generators and by increasing muscle tone of both extensor and flexor muscles. Excessive GABAergic basal ganglia efferents from the SNr to the MLR/PPN area are possibly involved in the gait failure and muscle tone rigidity (hypertonus) in PD.

Disclosures: K. Takakusaki: 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.; K.T. has received founding from Japanese Ministry of Education, Culture, Sports, Science and Technology ("Scientific Research-B; Grant # 2529001" and "Challenging Exploratory Research; Grant # 2365020. **T. Nozu:** None. **T. Okumura:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 270.09/XX3

Topic: D.15. Basal Ganglia

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NIH grant OD P51-OD011132 (previously NIH/NCRR grant P51RR000165) to the Yerkes National Primate Research Center

Title: Study of the physiology of the thalamostriatal system using optogenetics in nonhuman primates

Authors: *A. GALVAN, X. HU, Y. SMITH, T. WICHMANN;
Yerkes Res. Ctr. and Dept. Neurol., Emory Univ., ATLANTA, GA

Abstract: The caudal intralaminar nuclei, namely the centromedian/parafascicular complex (CM/PF), provide massive thalamic inputs to the striatum. In primates, the CM innervates mostly the sensorimotor region of the striatum (post-commissural putamen), while the PF sends afferents to the associative striatal territory (caudate nucleus). Both striatal projection neurons and interneurons receive synaptic inputs from the CM/PF. To understand how CM/PF influences striatal activity, we have begun to study the electrophysiological responses of striatal cells to light stimulation of channelrhodopsin (ChR2)-bearing CM/PF terminals in the monkey.
One monkey received recording chambers for access to CM/PF and the caudate/putamen. We injected 37 µl of AAV5-hSyn-ChR2-EYFP in 7 injection tracks to cover the extent of the CM/PF complex. After 9 weeks, the light stimulation experiments started. Optrodes (standard tungsten electrodes glued to 0.2 mm OD optical fibers) were introduced into the putamen or the caudate nucleus to light-activate ChR2s-positive terminals transfected via anterograde transport from the CM or PF injections, while we simultaneously recorded the extracellular activity of striatal neurons in response to light pulses (473 nm, 60-200 mW/mm2; trains of 10 pulses, 10 ms width, 20 Hz or 3 pulses, 10-30 ms width, 5 Hz).
We recorded 84 cells in the caudate nucleus, and 145 in the putamen. About 10% of caudate and 20% of putamen neurons responded significantly to the light stimulation, either during the train or within 0.5 ms after the end of the train. The responses were brief (<10 ms duration), not timed to the occurrence of individual light pulses, and appeared with a relatively long latency after the trains (>30 ms in many cases). While most responding neurons showed an increase in firing, 3 putamen cells decreased their activity following light stimulation. Both projection neurons and interneurons were among the responders. The long latencies to responses, and the recorded inhibitory effects of light stimulation upon striatal neurons, suggest that the physiological effects of CM/PF-striatal terminals stimulation are mediated in part through activation of the intrastriatal circuitry. Postmortem histological analysis will be performed to analyze the pattern of distribution and density of expression of ChR2s in CM/PF-striatal terminals across the caudate nucleus and putamen.

Disclosures: A. Galvan: None. **X. Hu:** None. **T. Wichmann:** None. **Y. Smith:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

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Program#/Poster#: 270.10/XX4

Topic: D.15. Basal Ganglia

Support: MH087473 NIMH

Title: Type III-Nrg1/ErbB4 signaling within the nucleus accumbens

Authors: *I. OBIORAH[1,2], L. W. ROLE[2,3], D. A. TALMAGE[1,2,3];
[1]Dept. of Pharmacol., [2]Centers for Nervous Syst. Disorders, [3]Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Nrg1-ErbB4 signaling has been implicated in the maintenance of synaptic plasticity, dendritic spine density, neuronal migration and survival, and GABAergic, dopaminergic, cholinergic and glutamatergic transmission. Type III-Nrg1 heterozygous mice show: (a) altered functional connectivity between the ventral hippocampus and the nucleus accumbens, and (b) deficits in behaviors in which the hippocampus-accumbens circuit plays a role. Based on these findings, we hypothesized that Nrg1-ErbB4 signaling plays an important role in synaptic transmission within accumbens circuits. Type III Nrg1 is expressed in neurons that project to the nucleus accumbens, whereas ErbB4 is expressed in the accumbens. In the early post-natal period, a subset of striatal medium spiny neurons, parvalbumin-positive neurons and cholinergic interneurons express ErbB4. Striatal ErbB4 expression decreases as animals mature and in adults becomes restricted to parvalbumin-positive interneurons. In order to begin to understand the role of Nrg1-ErbB4 signaling in excitatory transmission in the nucleus accumbens, we quantified the levels of AMPA receptor subunits in the accumbens of Type III Nrg1 heterozygous and ErbB4 knockout mice. Our major findings are: (a) GluA1 alternative splicing changes as animals mature. At birth 95-100% of GluA1 transcripts contain the flip exon, at P21 70% of GluA1 transcripts contain the flip exon and in adults 50% do. (b) Type III Nrg1 heterozygotes have alterations in the developmental profile of GluA1 splice variants; the transition from all flip to a mix of flip and flop containing transcripts seen in wild type animals is accelerated in the Nrg1 heterozygotes. The splicing of GluA1 transcripts in ErbB4 knockout mice is similar to WT profile. Thus, although ErbB4 expression in striatal MSNs and GluA1 splicing change in parallel during maturation, the regulation of GluA1 splicing by Type III Nrg1 appears to be ErbB4 independent. On going studies are to investigate the signaling mechanisms by which Type III-Nrg1 mediates splicing of GluA1 transcripts.

Disclosures: I. Obiorah: None. **L.W. Role:** None. **D.A. Talmage:** None.

Poster

270. **Basal Ganglia: Physiology**

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Topic: D.15. Basal Ganglia

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Title: Functional connectivity between the cerebellum and basal ganglia in a songbird

Authors: L. PIDOUX, C. LEVENES, D. DUBAYLE, *A. LEBLOIS;
CNRS / Univ. Paris Descartes, Paris, France

Abstract: Human speech is a complex sensorimotor skill and vocal learning is one of the most striking cognitive abilities of the brain. As many other complex motor skills, vocal learning involves the basal ganglia (BG)-thalamo-cortical network and the cerebello-thalamo-cortical network in humans. While the BG and cerebellar sub-cortical loops have been shown to interact at least through two different pathways in mammals, the role of their interaction during sensorimotor learning, and in particular during vocal learning, remains undetermined. Songbirds are one of the few accessible animal models for vocal learning, as they have a specialized portion of their BG-thalamo-cortical circuitry dedicated to song learning. Additionally, a cerebellar projection to the thalamic region adjacent to the song-related thalamic nucleus receiving BG input (DLM) suggests that the BG and cerebellum may interact during song learning. However, very little is known about song-related circuits in the cerebellum, or about a putative cerebellar function in song learning. We are studying the interactions between BG and cerebellar sub-cortico-cortical loops involved in avian song learning.
First, we confirmed the anatomical connection from the deep cerebellar nuclei to a thalamic region adjacent to the song-related BG-target thalamic nucleus DLM, which in turn projects to the song-related basal ganglia nucleus Area X, as shown previously. More importantly, we show that this anatomical pathway is functional. Indeed, electrical stimulation in the deep cerebellar nuclei consistently evoked fast excitatory responses in pallidal neurons located in the song-related nucleus Area X, with relatively short latencies consistent with a disynaptic pathway between the two structures. Finally, we also explored putative anatomical pathways linking the song control areas in the forebrain to the cerebellum.
Overall, our results suggest that the cerebellum is involved in song learning through its interaction with the song-related BG-thalamo-cortical loop in songbirds, providing a unique model to study the interactions between cerebellum and basal ganglia during sensorimotor learning.

Disclosures: L. Pidoux: None. **C. Levenes:** None. **A. Leblois:** None. **D. Dubayle:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: D.15. Basal Ganglia

Title: Effects of caged-dopamine photolysis on spike-timing dependent long-term depression in mice striatal neurons

Authors: *M. OCHI-SHINDOU, T. SHINDOU, J. R. WICKENS;
Neurobio. Res. Unit, Okinawa Inst. of Sci. & Tech., Okinawa, Japan

Abstract: Recent evidence suggests that the temporal association of cortical and striatal neuron activity modifies corticostriatal synaptic efficacy. Dopamine (DA) has been suggested to play a crucial role in the corticostriatal synaptic plasticity. We have studied spike-timing dependent plasticity (STDP) and found that spike-timing dependent long-term depression (t-LTD) was induced by prepost protocols in D1R-expressing projection neurons in the striatum. Here we tested the hypothesis that DA plays a crucial role in corticostriatal t-LTD using the rapid application techniques by caged-dopamine photolysis. Whole cell recording in slice preparations obtained from adult BAC transgenic mice that selectively express GFP in D1R-expressing cells was performed in this study. Photolysis of CNB-caged DA by xenon flash induced DA release with a peak concentration of approximately 0.5 µM, estimated by fast-scan cyclic voltammetry methods. The photolysis of caged-DA elicited large hyperpolarisation in DA neurons in substantia nigra, which was selectively blocked by a D2R antagonist, confirming that uncaged-DA acts on DA receptors in our experimental conditions. Then the effects of photolysis of caged-DA on corticostriatal t-LTD were investigated. The photolysis was applied at 300 ms prior to, 0 msec, 300 msec, or 2 seconds after the pairing protocols. Of these timing conditions, the photolysis at 2 seconds after the pairing blocked the t-LTD, whereas there were no effects of other conditions on t-LTD. We suggest that the timing of DA release could be an important factor to modulate the corticostriatal synaptic plasticity.

Disclosures: M. Ochi-Shindou: None. **T. Shindou:** None. **J.R. Wickens:** None.

Poster

270. **Basal Ganglia: Physiology**

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Parkinson's Disease Foundation PDF-IRG-1101

Title: Long-term potentiation of external globus pallidus-subthalamic nucleus synapses following activation of motor cortical inputs

Authors: H.-Y. CHU, *M. D. BEVAN;
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Abstract: The frequency and pattern of activity in the subthalamic nucleus (STN) are intimately related to motor function and dysfunction and are influenced in large part by the STN's major glutamatergic afferent, which arises from the motor cortex and its major GABAergic afferent, which arises from the external globus pallidus (GPe). In order to understand how the balance of cortical excitation and pallidal inhibition is regulated in the STN, the impact of optogenetic activation of motor cortical inputs on GPe-STN inputs was studied in *ex vivo* brain slices using patch clamp recording. Repeated optogenetic activation (300 ms trains of 50 Hz stimulation repeated 30 X at 0.2 Hz) of motor cortical inputs led to heterosynaptic long-term potentiation (LTP) of GPe-STN inputs. IPSC1 potentiated by 41 ± 7%. LTP was associated with a reduction in the ratio of IPSC2:IPSC1 (from 0.84 ± 0.01 to 0.72 ± 0.02) and a reduction in the coefficient of variation of IPSC1 (0.12 ± 0.02 to 0.06 ± 0.01), consistent with an increase in the initial probability of GPe-STN transmission. LTP was mimicked by bath application of 50 μM NMDA (43 ± 7%) and prevented by blockade of NMDA receptors (NMDARs) with 50 μM D-APV or chelation of postsynaptic Ca^{2+} with 20 mM BAPTA applied via the patch pipette. LTP was also prevented by inhibition of nitric oxide synthase with 100 μM L-NAME or scavenging of nitric oxide (NO) with 30 μM PTIO or inhibition of guanylate cyclase (GC) with 10 μM ODQ or inhibition of PKG with 1 μM KT5823. LTP was not induced by application of NO donors 100 μM SNAP or 100 μM spermine NONOate, suggesting that NO/GC/cGMP/PKG signaling is necessary but not sufficient for LTP. Together these data suggest that motor cortical inputs activate postsynaptic NMDARs leading to the production of NO and activation of GC/cGMP/PKG signaling in GPe-STN axon terminals, which mediates an increase in the probability and strength of GPe-STN transmission. Following the chronic loss of dopamine GPe-STN synapses proliferate leading to a profound increase in the strength of GPe-STN transmission. We are therefore investigating whether cortical excitation can ultimately trigger synaptic remodeling of GPe-STN inputs.

Disclosures: H. Chu: None. **M.D. Bevan:** None.

Poster

270. **Basal Ganglia: Physiology**

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Topic: D.15. Basal Ganglia

Support: Clarendon Fund

Parkinson's UK

Title: The relative role of thalamic and cortical afferents in driving cholinergic interneuron-evoked striatal dopamine release

Authors: *P. KOSILLO[1], S. THRELFELL[2], S. CRAGG[2];
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Abstract: Acetylcholine (ACh) and dopamine (DA) are two striatal neuromodulators, critical for the basal ganglia (BG) circuits. Both neurotransmitters are important for the balance between direct and indirect BG pathways, thereby regulating normal motor output. Further, ACh and DA extensively modulate striatal function, and hence are involved in the processes of action selection, learning, habit formation and addiction.

ACh and DA were traditionally conceptualized to co-exist antagonistically, as the two systems reciprocally inhibit each other's release. However, this hypothesis is oversimplified in the light of data showing dynamic gating of striatal DA release probability by ACh and optogenetic paradigms demonstrating an unprecedented level of ACh-DA co-operativity. The latter experiments established that synchronous activation of a small population of cholinergic interneurons (ChIs) both in vivo and in vitro is capable of directly driving DA release from striatal dopaminergic terminals via ACh acting at nicotinic ACh receptors. Crucially, this local striatal mechanism for ChI-evoked DA release bypasses parent cell body activation of DA neurons residing in subtantia nigra pars compacta and ventral tegmental area. The physiological context for this direct axon-axonic action of ACh on striatal DA terminals remains incompletely understood. This project aims to identify and characterize which striatal inputs are capable of driving ChI-evoked DA release.

Two major glutamatergic inputs known to make synaptic contacts with and regulate the firing rate of striatal ChIs originate from somatosensory cortex and caudal intralaminar thalamus. In this study we use fast-scan cyclic voltammetry to monitor real-time striatal DA release in combination with optogenetic activation of the cortical or thalamic striatal terminals, following transfection with ChannelRhodopsin2 of the two regions of interest in CamK2a-cre transgenic mice. Experiments performed in acute brain slices aim to investigate the relative roles of these projections in driving ChI-mediated DA release. Preliminary data suggests that somatosensory cortical afferents, as well as inputs from caudal intralaminar thalamus are capable of driving ChI-evoked DA release. However, the two pathways differ in the relative contribution from NMDA and AMPA ionotropic glutamate receptors and the dynamic timecourse of DA re-release. These

data highlights previously unappreciated role of thalamic and cortical afferents in control of local striatal circuits regulating DA release and adds a new level of complexity in understanding BG function.

Disclosures: P. Kosillo: None. **S. Threlfell:** None. **S. Cragg:** None.

Poster

270. **Basal Ganglia: Physiology**

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Topic: D.15. Basal Ganglia

Support: NIMH Grant MH079276

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Title: Heterosynaptic ltp of identified gabaergic inputs onto dopaminergic neurons

Authors: *D. SIMMONS, C. PALADINI;
Univ. of Texas At San Antonio, San Antonio, TX

Abstract: The net output of a dopaminergic neuron depends on the balance between excitatory and inhibitory inputs onto the cell, and tonic imbalances of these inputs persistently alter the release of dopamine. Inhibitory, GABAergic inputs onto a dopaminergic neuron in the ventral tegmental area exhibit long term potentiation (LTPGABA) following LTP-inducing activity of glutamatergic inputs onto the same cell. Therefore, a heterosynaptic balancing mechanism exists whereby high-frequency activity that increases glutamatergic synaptic weight also increases GABAergic synaptic weight. It is unknown, however, which GABAergic and glutamatergic inputs participate in LTPGABA. Rostromedial Tegmental nucleus (RMTg) and Nucleus Accumbens (NAc) are the largest GABAergic projections to ventral tegmental area dopaminergic neurons. The Pedunculopontine (PPN) and Subthalamic (STN) nuclei are glutamatergic inputs to dopaminergic neurons whose activity may induce LTPGABA. We infected each of these nuclei with the light-activated protein, channelrhodopsin, and found that LTPGABA enhanced both RMTg- and NAc-specific IPSCs. High-frequency stimulation of projections from PPN, but not STN, induced LTPGABA from IPSCs evoked by electrical stimulation. IPSCs from RMTg inputs, but not NAc inputs, exhibited LTPGABA following high-frequency stimulation of PPN projections. In conclusion, RMTg synapses use LTPGABA to counterbalance LTP at PPN synapses onto dopaminergic neurons. Future studies will determine which glutamatergic input is counterbalanced by LTPGABA at NAc synapses.

Disclosures: D. Simmons: None. **C. Paladini:** None.

Poster

270. **Basal Ganglia: Physiology**

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IMPULSA03-UNAM

E. Galarraga and J. Bargas

Title: Cholinergic modulation of corticostriatal synaptic response of direct and indirect projection neurons

Authors: *M. B. PEREZ-RAMIREZ, D. TAPIA, J. BARGAS, E. GALARRAGA; INSTITUTO DE FISIOLOGIA CELULAR, MEXICO D.F., Mexico

Abstract: Striatal projection neurons (SPNs) receive monosynaptic glutamatergic inputs from diverse cortical areas. Monosynaptic contacts between pyramidal neurons and striatal neurons of the direct and indirect pathways (dSPNs and iSPNs), as well as with striatal interneurons, have been described based on the small variation in latency of the synaptic events. High levels of acetylcholine (ACh) can be found in the neostriatum.
SPNs activity can be modulated by Ach trough M_1 muscarinic acetylcholine receptors (mACh-R M_1). It is well known that this receptor type modulates several currents, one of which is the potassium Kv7 current. Yet, the cholinergic modulation of the synaptic response in the dSPN and iSPNs is unknown. Based on the electrophysiological differences that distinguished these projection neurons, with whole cell recordings, we studied the cholinergic modulation by activating (with 1-10 μM muscarine) or inhibiting (with 5nM of the muscarinic toxin MT-7) the mACh-R M_1. We also blocked the Kv7 current (with 20μM XE991). Here is shown that cortical input is received by the cholinergic interneurons and by the dSPNs and iSPNs with different latencies: TAN 1.24 ± 0.11 ms; dSPN 2.37 ± 0.22 ms; iSPN 2.82 ± 0.22 ms.
We confirm in Wistar rats and transgenic BAC-D1 and BAC-D2 mice, that the synaptic corticostriatal responses are different in the SPNs: in dSPNs, the responses are more prolonged and evoke more action potentials, while in iSPNs the synaptic responses are briefer and exhibit

intrinsic autoregenerative responses (Flores-Barrera et al, 2010). When mACh-R M_1 was blocked with the M_1 muscarinic toxin MT-7, a decrease in the area under the synaptic response was observed: dSPN 18% and in iSPN 22%. On the other hand, when M_1 receptors were activated with muscarine, there was an increase in the area under the synaptic response in both dSPNs and iSPNs, 14% and 28% respectively. Similar results were obtained after XE991, a Kv7 channel blocker. It is concluded that cholinergic modulation through Kv7 channels, is important to reduce synaptic facilitation in SPNs and to decrease the generation of dendritic autoregenerative calcium potentials in iSPNs.

Disclosures: M.B. Perez-Ramirez: None. **D. Tapia:** None. **J. Bargas:** None. **E. Galarraga:** None.

Poster

270. **Basal Ganglia: Physiology**

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Topic: D.15. Basal Ganglia

Title: Optogenetic inhibition of the primate nigro-collicular connection and its effects on saccadic eye movements

Authors: H. F. KIM[1], M. YASUDA[1], E. S. BOYDEN[2], *O. HIKOSAKA[1];
[1]Lab. Sensorimotor Res., Natl. Eye Inst., NIH, BETHESDA, MD; [2]MIT, Cambridge, MA

Abstract: As our first step toward the primate optogenetics, we chose to manipulate an output connection of the basal ganglia: the inhibitory connection from the substantia nigra pars reticulata (SNr) to the superior colliculs (SC). The nigro-collicular connection controls the initiation of saccadic eye movements. This was demonstrated by antidromic activation of SNr neurons by electrical stimulation of SC and reversible inactivation of SNr neurons by local injection of a GABA agonist (muscimol) (Hikosaka and Wurtz, 1983 & 1985).
We expressed Archaerhdopsin (ArchT), a light-driven proton pump, in the moneky's SNr to inhibit action potentials by optical stimulation (O-STIM). To this end, we injected AAV8 vector containing the ArchT-GFP gene selectively into the SC-projecting region of SNr in one monkey. Beginning 2 months after the virus injection, we performed three experiments using a custom-made optrode to deliver 532 nm laser.
1. Effects of O-STIM in SNr on the spike activity of SNr neurons.
All SNr neurons fire spontaneously with high frequencies. Most of the SNr neurons were suppressed during the O-STIM (duration: 500-1000 ms), followed by a rebound excitation. This

suggests that ArchT was expressed and functional in SNr neurons. Some SNr neurons, however, were activated during the O-STIM, presumably through collateral connections among SNr neurons.
2. Effects of O-STIM in SC on the spike activity of SC neurons.
A majority of pre-saccadic neurons in SC were activated during the O-STIM, followed by a rebound inhibition. This result indicates that ArchT expressed in SNr neurons was carried along their axons to their terminals in SC, on which the O-STIM acted to reduce the SNr-induced inhibition on SC neurons.
3. Effects of O-STIM in SC on saccadic eye movements.
The monkey performed a memory-guided saccade task with 8 directions. The O-STIM was delivered after a go-signal (i.e., fixation point offset) (duration: 500 ms) on randomly chosen trials. The end points of memory-guided saccades were shifted weakly but systematically by the O-STIM: away from the center for the directions close to the response fields of nearby SC neurons, and toward the center for the opposite directions.
Our data indicate that the optogenetic technique using ArchT has a potential to overcome the previous limits of the primate circuit study.

Disclosures: H.F. Kim: None. **O. Hikosaka:** None. **M. Yasuda:** None. **E.S. Boyden:** None.

Poster

270. **Basal Ganglia: Physiology**

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Topic: D.15. Basal Ganglia

Support: Swedish Research Council

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Title: Synaptic activation of dendritic plateaus in medium spiny neurons - role of GABAergic inputs

Authors: *K. DU[1], J. HELLGREN KOTALESKI[2];
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Abstract: Striatum is the entrance to the basal ganglia, gating the information from cortex and thalamus. Medium spiny neurons (MSN) are principal neurons in striatum and play a key role in processing the cortical and thalamic inputs. In vivo experiments have shown that the membrane potential of MSNs fluctuates between a hyperpolarized “down" state and a depolarized “up" state, and the transition from down-state to up-state is likely driven by synchronized inputs from cortex (Stern et al., 1998). A recent in vitro experimental study (Plotkin et al., 2011) showed that synchronized activation of clustered spines in the distal dendrites of MSNs can give rise to long-lasting plateaus in the dendrites and soma simultaneously, indicating that synaptically driven dendritic plateaus might be essential for understanding how MSNs process synchronized inputs from cortex.

The striatal circuit is a predominantly inhibitory GABAergic network, which includes lateral “feedback" inhibition among MSNs and a powerful “feedforward" inhibition from fast-spiking interneurons (FS) to MSNs. To investigate how these GABAergic inputs affect dendritic plateaus in MSNs, we have built a biophysically detailed MSN model, which contained complex dendritic morphology and a large array of ion channels (Paille, et al., 2013) verified by experimental data. This MSN model can faithfully reproduce the phenomenon of dendritic plateaus as shown in Plotkin et al. (2011). The simulations showed that both feedforward and feedback inhibition have weak influence on dendritic plateau formation when the MSN model rests at -80 mV, as in the in vitro down state. Interestingly, when the MSN model fluctuates around -65 mV, as during in vivo-like conditions, the GABAergic inputs can greatly dampen the dendritic plateau, in particular when the reversal potential of GABA is set to -75 mV. Our simulations thus suggest that the GABAergic effects on dendritic plateau formation in MSNs are sensitive to experimental conditions, in particular the resting membrane potential and the reversal potential of GABA.

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Plotkin, J. L., Day, M., & Surmeier, D.J. Synaptically driven state transitions in distal dendrites of striatal spiny neurons. Nat Neurosci. 14(7): 881-888 (2011).

Paille, V., Fino,E., Du,K., Morera-Herreras, T., Perez, S., Hellgren Kotaleski,J., and Venance,L. GABAergic circuits control spike-timing-dependent plasticity. Journal of Neuroscience, accepted.

Disclosures: K. Du: None. **J. Hellgren Kotaleski:** None.

Poster

270. **Basal Ganglia: Physiology**

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Title: Consequences caused in the neuronal activity of the putamen by an enhancer stimulus

Authors: *M. MONTES LOURIDO[1], A. F. VICENTE[2], M. A. BERMUDEZ[2], M. C. ROMERO[2], R. PEREZ[3], F. GONZALEZ[4];
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Abstract: The putamen is part of the basal ganglia and its function is mainly related to motor coordination. However the hypothesis of this work assumes that neural activity into the putamen nucleus is to be modulated by the different components of the task, the presence or absence of a stimulus enhancer, motor act, and the presence of the reward. In our study we used a visual stimulus (silhouette of a white square) as enhancer of another visual stimulus (abstract image). We used four abstract images: two images meant 'Yes' and were associated with obtaining reward and two images meant 'Non' and were associated with no reward. In the images 'Yes' the animal had to press a lever to obtain a reward, in the images 'No' the animal could not press the lever. In some trials, featuring the 'Enhancer' after the appearance of the images. The 'Enhancer' indicated the existence of double reward in Images 'Yes' and more waiting between trials if it presented before the images 'Non'. We recorded the activity of neurons in the putamen of a monkey (Maccaca mulatta) trained to perform this visuomotor task that requires the execution of a movement and retention of a move to the presentation of different images. We used an ANOVA statistical test to study neuronal activity during task execution. Of all the recorded neurons, 8% increased their activity after the presentation of the 'Enhancer' (ANOVA, $p < 0.05$), 61% increased their activity around the lever down (ANOVA, $p < 0.05$) and 31% increased their activity after the presentation of the reward (ANOVA, $p < 0.05$). These results indicate that the putamen is related to stimulus processing relevant to the task, with the motor action and reward processing.

Disclosures: M. Montes Lourido: None. **A.F. Vicente:** None. **M.A. Bermudez:** None. **M.C. Romero:** None. **R. Perez:** None. **F. Gonzalez:** None.

Poster

270. **Basal Ganglia: Physiology**

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NIH Grant DA030530

Title: Regulation of NMDAR-dependent burst firing in midbrain dopaminergic neurons by glycine release from glia

Authors: *G. M. BEAUDOIN, III, C. A. PALADINI;
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Abstract: The firing activity of dopaminergic neurons reflects the integration of information from complex environmental stimuli via afferents. Activation of glutamatergic afferents of dopaminergic neurons induces burst firing, however the molecular mechanisms regulating induction of burst firing are unknown. While activation of NMDA-type receptors regulates burst firing, we now find that glycine release from glia controls induction of NMDAR-mediated currents required for burst induction. Using slice electrophysiology, treatment with glycine can increase NMDAR-mediated currents and induces bursts with more spikes. Rather than glycine coming from glycinergic afferents, we suggest glycine comes from glia as inhibition of the glial glycine transporter, GLYT1, decreased both NMDAR-mediated currents and burst induction. This effect is specific for glycine as inhibition of glutamate transporters increases NMDAR-mediated currents and induces stronger bursts. Mechanistically, we find glycine regulates both activation of extrasynaptic NMDARs and conductance of synaptic NMDARs. By inhibiting either synaptic or extra-synaptic NMDARs, our results show that burst induction requires both sources – synaptic and extra-synaptic – of NMDAR-mediated currents. Glycine regulation of synaptic NMDARs is due to a unique configuration of these receptors containing the NR3 subunit with the traditional NR1 and NR2B or D subunits. The NR3 subunit binds glycine, increasing the dependence of this triheteromeric NMDAR on glycine. Together, these mechanisms regulate the induction of NMDAR-mediated currents and burst firing in dopaminergic neurons, and suggest this mechanism as a coincidence detector. Supported by grants from NIH-NIDA and NIMH.

Disclosures: G.M. Beaudoin: None. **C.A. Paladini:** None.

Poster

270. **Basal Ganglia: Physiology**

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Topic: D.15. Basal Ganglia

Support: 1R01NS079750-01A1

Title: Cerebellar modulation of corticostriatal plasticity

Authors: *C. H. CHEN, E. ARTEAGA-BRACHO, K. KHODAKHAH;
Albert Einstein Col. of Med., Bronx, NY

Abstract: The cerebellum and basal ganglia are structures critical for the control of movement. We have observed in awake, freely moving mice that optogenetic or electrical stimulations of the cerebellum can elicit short latency (8-12 ms) responses in ≈50% of cells in the dorsolateral striatum. These responses are abolished upon inactivation of thalamic intralaminar nuclei and remain with cortical inactivation, indicating that they are routed through the thalamus. This data corroborates published anatomical tracing data, and also provides functional evidence for a robust and short-latency pathway mediating subcortical motor communication between the cerebellum and basal ganglia. In addition to allowing rapid coordination of the two structures, we wondered whether this pathway provides the cerebellum with the ability to modulate synaptic plasticity and thus learning in the basal ganglia. A major site of plasticity in the basal ganglia is the corticostriatal synapse. It has been shown in vitro that high frequency stimulation (HFS) of the corticostriatal synapse results in its long term depression (LTD). However, if the striatal neuron is depolarized during HFS the sign of the plasticity is reversed and the corticostriatal synapse undergoes long term potentiation (LTP) rather than LTD. We postulated that the short latency cerebellar activation of striatal neurons can effectively depolarize the striatal neurons and thus modulate the direction of corticostriatal plasticity. To test this hypothesis we first confirmed that in awake-freely moving mice HFS of the motor cortex resulted in LTD of the corticostriatal synapse. We then paired the HFS of the cortex with simultaneous HFS of the cerebellum. We found that under these conditions the corticostriatal synapse showed LTP, rather than LTD, thus reversing direction. This data demonstrates that cerebellum can regulate the direction and perhaps extent of plasticity at corticostriatal synapses, thus enriching the nature of interactions between these two structures.

Disclosures: C.H. Chen: None. **K. Khodakhah:** None. **E. Arteaga-Bracho:** None.

Poster

270. **Basal Ganglia: Physiology**

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NIH-NINDS P50NS047085

CHDI

Title: Intrinsic and synaptic properties of subthalamic nucleus neurons in BACHD mice

Authors: *J. F. ATHERTON, E. L. MCIVER, M. D. BEVAN;
Physiol., Northwestern Univ., CHICAGO, IL

Abstract: The BACHD mouse model of Huntington's disease expresses full-length human mutant huntingtin and is associated with abnormal intrinsic and synaptic properties in cortical and striatal neurons together with neuronal degeneration and motor dysfunction. The subthalamic nucleus (STN) is a key element of cortico-basal ganglia thalamocortical circuitry but its properties have not been thoroughly characterized in the BACHD model. The aim of this project was therefore to determine whether the STN exhibits abnormalities that contribute to the BACHD phenotype. The intrinsic and synaptic properties of STN neurons in BACHD and wild type (WT) litter mate control mice were compared at pre-symptomatic (<2 months) and symptomatic (6 months) ages using patch clamp recording of *ex vivo* brain slices.
At 6 months (but not at <2 months) autonomous STN activity was reduced in its frequency and precision in BACHD (WT: freq = 8.4 ± 6.2 Hz, CV = 0.46 ± 0.32, n = 65; BACHD: freq = 5.2 ± 5.0, CV = 0.64 ± 0.46, n = 77) with 0% of WT and 16% of BACHD neurons inactive. Autonomous STN activity in BACHD was restored to WT levels by application of the K_{ATP} channel blocker gibenclamide (100 nM) (WT: freq = 14.6 ± 4.4 Hz, CV = 0.17 ± 0.09, n = 8; BACHD: 12.9 ± 3.9 Hz, CV = 0.17 ± 0.10, n = 9). Autonomous activity in WT neurons was persistently disrupted after preincubation in 12.5 μM NMDA for 60 mins (control: freq = 5.7 ± 3.3 Hz, CV = 0.38 ± 0.25, n = 24; NMDA: freq = 1.4 ± 2.8 Hz, CV = 1.08 ± 0.80, n = 14) or by acute application of the NO donor SNAP (100 μM) (control: freq = 5.6 ± 3.6 Hz, CV = 0.18 ± 0.10, n = 7; SNAP: freq = 3.0 ± 2.8 Hz, CV = 0.90 ± 0.88, n = 7) but was restored to control levels by K_{ATP} channel blockade. Together these data imply that NMDA-NOS-NO-GC-cGMP-PKG signaling-mediated upregulation of K_{ATP} channels may underlie disrupted autonomous STN activity in BACHD similar to that observed in Parkinson's disease models.
The frequency of mEPSCs in BACHD was greater at <2 months (WT: 0.17 ± 0.12 Hz, n = 13; BACHD = 0.82 ± 0.43, n = 11) but similar at 6 months (WT: 0.22 ± 0.21 Hz, n = 14; BACHD = 0.14 ± 0.16, n = 9). mEPSCs recorded at -70 mV were largely mediated by AMPA receptors (Rs) and exhibited similar amplitudes and kinetics in BACHD and WT at both time points. mEPSCs recorded at 40 mV were largely mediated by NMDARs and exhibited prolonged decay time constants in BACHD versus WT at <2 (WT: 44.1 ± 13.2 ms, n = 14; BACHD = 84.3 ± 40.6 ms,

n = 10) and 6 months (WT: 40.8 ± 20.8 ms, n = 7; BACHD = 82.0 ± 27.1 ms, n = 6). Together these data suggest that disruption of autonomous STN
activity may be related to abnormalities in STN NMDARs in the BACHD model.

Disclosures: J.F. Atherton: None. **E.L. McIver:** None. **M.D. Bevan:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 270.23/YY5

Topic: D.15. Basal Ganglia

Support: NIH Grant K99 NS076524

Title: Identification and characterization of neuronal subpopulations in the GPe using transgenic mice

Authors: K. J. MASTRO[1], R. S. BOUCHARD[2], C. WINKLER[2], *A. H. GITTIS[3,4];
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Abstract: Neurons in the external segment of the globus pallidus (GPe) participate in basal ganglia circuits that are critical for motor function. Heterogeneity of neuronal populations within the GPe has been described using various anatomical and functional criteria, but a rigorous classification scheme has yet to be established. Using transgenic mice, we define two non-overlapping cell populations in the GPe. Neurons labeled in Lhx6 transgenic mice make up 27% of GPe neurons and are more prominent in the medial portion of the GPe. In contrast, neurons labeled in PV transgenic mice make up 34% of GPe neurons and are more prominent in the lateral portion of the GPe. Anatomical targets of each GPe cell type were identified by expressing EYFP under viral transfection in either Lhx6-cre or PV-cre mice. As expected, axons from both cell types were found in downstream and upstream basal ganglia nuclei: subthalamic nucleus, susbstantia nigra, and striatum. Intriguingly, axons from both cell types were found in the reticular nucleus of the thalamus, and in PV-cre mice, a prominent collection of axons was also observed in a medial thalamic nucleus, likely the parafascicular nucleus. Electrophysiologically, PV neurons have lower input resistances and higher spontaneous and maximum firing rates than Lhx6 neurons. Combined, our data suggest that PV and Lhx6 neurons represent anatomically and physiological distinct neuronal populations in the GPe. These results provide a framework for the study of GPe circuits in basal ganglia function in both health and disease.

Disclosures: K.J. Mastro: None. **A.H. Gittis:** None. **R.S. Bouchard:** None. **C. Winkler:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 270.24/YY6

Topic: D.15. Basal Ganglia

Support: European Commission Grant Cogsystems FP7-250013

Ministero dell'Istruzione, dell'Universita` e della Ricerca Grant PRIN 2010, 2010MEFNF7_005

Title: Connectional evidence for multiple basal ganglia grasping loops in the macaque monkey

Authors: M. GERBELLA[1,2], E. BORRA[3], S. ROZZI[1,2], *G. LUPPINO[1,2];
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Abstract: It is largely agreed that different sets of functionally related cortical areas project to different parts of the striatum, which are at the origin of largely segregated basal ganglia-thalamocortical loops. Specifically, caudal, corticospinal motor areas (M1, SMA, and caudal premotor areas) are a source of partially overlapping projections to a large putaminal sector caudal to the anterior commissure (AC). This sector is at the origin of a "motor" loop involved in modulating the cortical motor output during the execution of voluntary movements. In contrast, the distribution of the striatal projections of frontal and parietal areas involved in selecting and organizing voluntary actions is still largely unknown. To address this issue, we analyzed the corticostriatal projections of the inferior parietal (AIP and PFG), ventral premotor (F5a and F5p), and ventrolateral prefrontal (intermediate 12r and rostral 46vc) areas forming a network (lateral grasping network) involved in selecting and controlling goal-directed hand actions, based on visual and memory-based information and on behavioral goals. The results, based on anterograde neural tracer injections (12 injections, 9 macaques) showed that after injections in all the studied areas but F5p, patches of dense labeled terminals were observed in two distinct putaminal zones located at different rostrocaudal levels. One zone was located in the mid-ventral part of the putamen and extended for about 4 mm from the AC in rostral direction, i.e. just rostral to the putaminal sector of the "motor" loop. The other zone was located in the caudalmost part of the putamen and extended for about 3 mm in rostrocaudal direction, ventral to the putaminal sector of the "motor" loop. Additional labeled zones were observed in other striatal sectors, varying in

location according to the injected area. Specifically, after injections in F5a, the labeling also involved the putaminal sector of the “motor” loop. In contrast, the corticospinal area F5p, which in the lateral grasping network represents the gateway for the access of signals related to the selection of hand motor acts to M1, differently from the other areas of the lateral grasping network, displayed projections mostly involving the putaminal sector of the “motor” loop. The present data indicate that areas of the lateral grasping network are a source of projections likely overlapping in two striatal zones distinct from the putaminal sector targeted by corticospinal motor areas. Accordingly, these data provide evidence for multiple grasping basal ganglia loops, possibly differentially involved in controlling goal-directed hand actions.

Disclosures: M. Gerbella: None. **E. Borra:** None. **S. Rozzi:** None. **G. Luppino:** None.

Poster

270. **Basal Ganglia: Physiology**

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Topic: D.15. Basal Ganglia

Support: FRS-FNRS

FMRE

ARC-FWB

IAP-Belgian Federal State

Title: FACS-array profiling identifies Ecto-5’ nucleotidase as a striatopallidal neuron-specific gene involved in striatal-dependent learning

Authors: *S. N. SCHIFFMANN, S. ENA, J.-F. DE BACKER, A. DE KERCHOVE D'EXAERDE;
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Abstract: The striatopallidal (STP) and striatonigral (STN) neurons constitute the main neuronal populations of the striatum. Despite the increasing knowledge concerning their involvement in multiple tasks associated to the striatum, it is still challenging to understand the precise differential functions of these two neuronal populations and to identify and study new genes involved in these functions. Here, we describe a reliable approach, applied on adult mouse brain to generate specific STP and STN neuron gene profiles. STP and STN neurons were identified in the same animal using the transgenic Adora2aCre-Z/EG mouse model combined with retrograde

labeling, respectively. Gene profiling were generated from FACS-purified neurons leading to the identification of new STP and STN neuron specific genes. Knockdown models based on Cre-dependent lentiviral vector were developed to investigate their function either in striatal or in STP neurons. Thereby, we demonstrate that ecto-5'-nucleotidase (NT5e) is specifically expressed in STP neurons and is at the origin of most of the extracellular adenosine produced in the striatum. Behavioral analysis of striatal and STP neuron knockdown mouse models as well as NT5e knockout mice demonstrates the implication of this STP neuron enzyme in motor learning. and therefore highlights the central role of the neuronal NT5e associated to adenosine receptors in striatum-dependent learning.

Disclosures: S.N. Schiffmann: None. **A. de Kerchove d'Exaerde:** None. **S. Ena:** None. **J. De Backer:** None.

Poster

270. **Basal Ganglia: Physiology**

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Topic: D.15. Basal Ganglia

Support: Howard Hughes Medical Institute

NIH Grant NS046579

Nancy Lurie Marks Family Foundation postdoctoral fellowship

Title: Midbrain dopaminergic neurons acquire GABA through plasma membrane uptake, not synthesis

Authors: *N. X. TRITSCH[1], W.-J. OH[2], C. GU[2], B. L. SABATINI[1];
[1]Dept of Neurobio., Harvard Med. School, HHMI, Boston, MA; [2]Dept of Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Synaptic transmission between dopamine-containing neurons in the midbrain and target neurons in the striatum plays an essential role in the selection and reinforcement of voluntary movements based on contextual information. Although the importance of dopamine release for these processes is well established, recent evidence indicates that dopaminergic neurons additionally influence striatal circuit function through co-release of fast-acting neurotransmitters along with dopamine. In particular, we recently showed that nigrostriatal afferents in mice exert a rapid and potent inhibitory influence on the activity of striatal projection

neurons (SPNs) by releasing a neurotransmitter that activates $GABA_A$ receptors (Tritsch *et al.*, 2012). We now extend these findings by showing that this phenomenon also applies to mesolimbic afferents, and by providing additional functional evidence that the released neurotransmitter is GABA. Surprisingly, we did not detect the GABA synthetic enzymes GAD65 or GAD67 in dopaminergic neurons of the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) using either *in situ* hybridization or immunolabeling in transgenic mice. However, VTA/SNc dopaminergic neurons expressed mRNA for the membrane GABA transporters mGAT1 (*Slc6a1*) and mGAT4 (*Slc6a11*) and pharmacological antagonism of these transporters prevented GABA release from these cells. Thus, these findings indicate that GABA co-release is a general feature of midbrain dopaminergic neurons that depends on membrane uptake of GABA from the extracellular milieu but not on GABA synthesis. This unusual mechanism may confer dopaminergic neurons the flexibility to differentially control GABAergic transmission locally across its extensive axonal arbors.

Disclosures: N.X. Tritsch: None. **W. Oh:** None. **C. Gu:** None. **B.L. Sabatini:** None.

Poster

270. **Basal Ganglia: Physiology**

Location: Halls B-H

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Program#/Poster#: 270.27/YY9

Topic: F.03. Motivation and Emotion

Support: Banting Postdoctoral Fellowship

NIDA P01 DA008227

Title: Chronic pain reduces motivation through depression of the indirect pathway

Authors: *N. SCHWARTZ, S. JURADO, B. K. LIM, J. S. POLEPALLI, R. C. MALENKA; Psychiatry and Behavioral Sci., Nancy Pritzker Laboratory, Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Comorbidities of chronic pain include fatigue and symptoms of depression. Despite the large impact these symptoms have on patients' quality of life, little is known about the neural circuit adaptations underlying their development. To address this issue we used the well-established models of CFA-induced chronic inflammatory pain, and of injury-induced neuropathic pain. In both of these models, we observed reduced motivation as measured by a decrease in operant responding for food reward. This reduction could not be explained by reduced mobility or a change in the value of the food reward. Ex vivo recordings from slices

revealed that both chronic pain models were accompanied by a depression of excitatory synaptic transmission in medium spiny neurons (MSNs) of the indirect pathway in the nucleus accumbens (NAc) core. Suppression of calcineurin dependent LTD in the NAc core rescued the chronic pain-induced deficit in motivation. These results suggest that chronic pain depresses excitatory synaptic transmission in the NAc core indirect pathway, and that this depression is required for the decreased motivation caused by these pain models. Thus, neural circuit adaptations within the NAc may contribute to the fatigue and energy malaise that often accompany chronic pain syndromes.

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

Location: Halls B-H

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Program#/Poster#: 271.01/YY10

Topic: D.17. Voluntary Movements

Support: NIH Grant NS036449

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NSF ENG-1137279 (EFRI M3C)

ONR MURI Award N00014-10-1-0072

Title: Non-linear dynamical analysis of EEG time series distinguishes patients with Parkinson's disease from healthy individuals

Authors: C. LAINSCSEK[1], M. E. HERNANDEZ[2], J. WEYHENMEYER[3], T. J. SEJNOWSKI[1], *H. POIZNER[4];
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Abstract: Objective, automatic methods of classifying, assessing, and tracking the progression of Parkinson's disease (PD) could prove of great use in the clinic. The pathophysiology of PD is known to involve altered patterns of neuronal firing and synchronization in cortical-basal ganglia circuits. Rather than using spectral-based methods, we used data models based on delay differential equations (DDE) as non-linear time-domain classification tools to distinguish resting

state electroencephalographic (EEG) recordings from PD patients on and off dopaminergic therapy and healthy individuals. Two sets of 50 1-s segments of 64-channel EEG activity were recorded from nine PD patients on and off medication and 9 age-matched controls. The 64 EEG channels were grouped into 10 clusters covering frontal, central, parietal, and occipital brain regions for analysis. DDE models were fitted to individual trials, and model coefficients and error were used as features for classification. Classification performance was measured using 3-fold cross validation across subjects. We found that even short segments of resting state EEG in PD patients and controls contained dynamical structure, and, moreover, that PD patients exhibited a greater dynamic range than controls. DDE model output on the means from one set of 50 trials provided nearly complete separation of PD patients off medication from controls: across brain regions, the area under the receiver operating characteristic curves, A′, varied from 0.97 - 1.0. For distinguishing PD patients on vs. off medication, classification performance (A′) ranged from 0.86 - 1.0 across brain regions. Moreover, the generalizability of the model to the second set of 50 trials was excellent, with A′ ranging from 0.74 - 0.92 across brain regions for controls vs PD off medication, and from 0.62-0.82 for PD on medication vs off. Finally, model features significantly predicted individual patients' motor severity, as assessed with standard clinical rating scales.

Disclosures: C. Lainscsek: None. **M.E. Hernandez:** None. **J. Weyhenmeyer:** None. **T.J. Sejnowski:** None. **H. Poizner:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

Location: Halls B-H

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Topic: D.17. Voluntary Movements

Support: Howard Hughes Medical Institute

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NSF ENG-1137279 (EFRI M3C)

NSF Grant SBE-0542013

Title: Non-linear dynamical classification of short time series of the Rossler system in high noise regimes

Authors: C. LAINSCSEK[1,2], M. E. HERNANDEZ[3], J. WEYHENMEYER[5,1], H. POIZNER[3], *T. J. SEJNOWSKI[6,4];
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Abstract: Time series analysis with delay differential equations (DDEs) reveals non-linear properties of the underlying dynamical system and can serve as a non-linear time-domain classification tool. In a companion paper, we apply the DDE model developed here to classify short segments of encephalographic (EEG) data recorded from patients with Parkinson's disease and healthy subjects. Here global DDE models were used to analyze short segments of surrogate time series from a known dynamical system, the Rossler system, in high noise regimes.
Nine simulated subjects in each of two distinct classes were generated by varying the bifurcation parameter b and keeping the other two parameters (a and c) of the Rossler system fixed. Data segments of 512 samples were used. All choices of b were in the chaotic parameter range. We diluted the simulated data using white noise ranging from 10 dB to -20 dB signal-to-noise ratios (SNR). Structure selection was supervised by selecting the number of terms, delays, and order of nonlinearity of the model DDE model that best linearly separated the two classes of data. The distances d from the linear dividing hyperplane was then used to assess the classification performance by computing the area under the receiver operating characteristic (ROC) curve, A'. The selected model was tested on untrained data using 3-fold cross-validation.
DDEs were able to accurately distinguish the two dynamical conditions, and moreover, to quantify the changes in the dynamics. There was a significant correlation between the dynamical bifurcation parameter b of the simulated data and the classification parameter d from our analysis. This correlation still held for new simulated subjects with new dynamical parameters selected from each of the two dynamical regimes. Furthermore, the correlation was robust to added noise, being significant even when the noise was greater than the signal (SNR = -10 dB for single trials A' = 0.6 and for means over 50 trials A' = 0.9; SNR = -15 dB for means of 50 trials, A' = 0.75.
We conclude that DDE models may be used as a generalizable and reliable classification tool for even small segments of noisy data, if the data have an underlying nonlinear dynamical structure.

Disclosures: C. Lainscsek: None. **T.J. Sejnowski:** None. **M.E. Hernandez:** None. **J. Weyhenmeyer:** None. **H. Poizner:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

Location: Halls B-H

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Program#/Poster#: 271.03/YY12

Topic: D.17. Voluntary Movements

Support: NSF grant BCS-1153034

Title: Position-dependent modulation of digit forces: Temporal evolution of anticipatory and feedback control interactions

Authors: *Q. FU, M. SANTELLO;
Arizona State Univ., Tempe, AZ

Abstract: Previous studies have shown that humans can adjust digit forces to compensate for trial-to-trial variability in digit position, thus resulting in consistent task performance. However, it was not clear whether this digit force-position coordination was accomplished through feed-forward planning (i.e., position and forces are both planned before contact) or feedback corrections (i.e., making corrections after sensing actual digit positions). We speculated that motor commands for digit forces and position are initially planned in a feed-forward fashion but digit forces can be adjusted after making contact with the object if a discrepancy between actual and planned positions is perceived. We tested this hypothesis using a virtual reality (VR) environment consisting of two haptic devices and a monitor. Subjects' thumb and index finger were attached to the haptic devices and interaction forces between the digits and the virtual object were simulated. Subjects were initially trained to perform a two-digit unconstrained torque production task using both small and large virtual boxes (widths = 42 or 72 mm; S or L, respectively). Subjects were required to apply a torque (±90 Nmm) to the box to control a horizontal moving cursor to the left or right of the center position to catch a downward moving ball. They had to adapt their digit force as function of box width, i.e., small and large forces for L and S box width, respectively. After learning the task, subjects were tested with a random sequence of box widths (1) with (Test A) or (2) without (Test B) visual information about box width. Importantly, in Test A subjects could have planned manipulation forces prior to making contact with the box by using visual width cues. In contrast, in Test B subjects had to make corrections to their motor plan after contact if the actual box width did not coincide with the width they had planned for. Consistent with our hypothesis, we found that subjects exerted digit forces that matched actual box sizes regardless of whether visual cues about box width were available prior to contact. A closer examination of the time course of the forces revealed that force development was delayed in all cases for Test B condition. Specifically, the development of the digit forces appropriate for the current box width, hence the relative digit positions, was delayed about 120-180 ms after initial contact. We propose that digit position-dependent force modulation can be explained by integrating pre-contact feed-forward anticipation and post-contact feedback control.

Disclosures: Q. Fu: None. **M. Santello:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Support: Bioengineering Research Partnership Grant R01-NS050265 from the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health (NIH) and

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Title: Feature extraction from grip force for identification of sensorimotor control processes

Authors: *K. MOJTAHEDI[1], M. SANTELLO[2];
[1]Sch. of Biol. and Hlth. Systems Engineering,, [2]Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Phoenix, AZ

Abstract: Previous work on the control of grasping has focused on the phenomenon of bell-shaped grip force rates as a feature indicating feedforward force control. However, this feature has not been assessed quantitatively. Furthermore, it is possible that the time course of grip force may contain additional features that could provide insight into sensorimotor control processes. In this study, we addressed these questions by verifying two computational approaches to extract features from grip force rates generated between contact and the onset of object manipulation (i.e. object lift) in humans. One approach consisted of fitting a Gaussian function to grip force rate and quantifying the goodness of the fit using the root mean square error (RMSE). The second approach consisted of applying continuous wavelet transform (CWT), which is based on the correlation of the grip force rate signal with a Mexican hat function. For this approach, we used the features of signal-to-summation of signal and noise. We applied both approaches to analyze grip force in two grasping tasks: (1) grasping an inverted T-shaped object whose center of mass (right, center, or left) was changed across blocks of consecutive trials (24 subjects), and (2) grasping an object whose mass (light or heavy) was pseudo-randomly changed on a trial-to-trial basis (24 subjects). For both tasks, subjects were asked to grasp the object at either predetermined (constrained) or self-selected (unconstrained) locations. The RMSE approach revealed that grip force rates during constrained grasping were better fitted by a Gaussian function than unconstrained grasping ($p < 0.05$ for both experiments). The wavelet approach confirmed this observation as revealed by signal-to-summation of signal and noise analyses ($p < 0.05$ for both experiments). Both analytical approaches suggest that grip force control is

mediated by feedback-driven corrections when subjects can self-select digit contacts on an object, as opposed to feedforward force development for constrained contact points. Future work will examine the application of CWT on a broader variety of manipulation tasks to extract digit force features that might provide insight into neural control of grasping.

Disclosures: K. Mojtahedi: None. **M. Santello:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 271.05/ZZ2

Topic: D.17. Voluntary Movements

Support: NSF BCS-1153034

Title: Parallel processes underlie learning of skilled tool use: Retention and interference

Authors: *M. SANTELLO, Q. FU;
Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., TEMPE, AZ

Abstract: A novel tool can be used in multiple contexts each requiring different hand-object interactions. Previous studies have shown an interference effect when switching between two opposite manipulation tasks performed on the same object, i.e., a change of manipulation context. As the source of the interference remains unclear, we quantified the extent to which dexterous manipulation in different contexts had to be learned and recalled. Subjects (n = 44) were asked to lift and balance an L-shaped object. A torque (320 Nmm) had to be produced on the handle at object lift onset to compensate for the object's asymmetrical mass distribution and prevent tilt. The direction of the necessary compensatory torque (Tcom) depended on the context, i.e., orientation of the object, which could be changed by the subject by rotating the object 180 degrees about the vertical axis of the handle. Subjects performed four blocks of eight trials and had to rotate the object after each block. We measured Tcom at object lift onset to quantify anticipatory grasp control. Subjects started with a moderate error of |Tcom| =192.67 ± 12.61 Nmm (mean ± SE), but learned the task equally well in the first two blocks of both contexts (|Tcom| = 288.7 ± 6.78 Nmm and 293.7 ± 7.45 Nmm for the last 5 trials of block 1 (context A) and block 2 (context B), respectively). After the first two blocks, subjects were divided into 4 groups and took breaks of different durations (0, 10, 20, or 60 minutes) before performing the third block in which they were asked to retrieve the manipulation learned in context A. We found a retrograde interference on the first retrieval trial for the 0-min break group (|Tcom| = 122.74 ± 15.22 Nmm). Most importantly, a significant effect of break duration

was found on the strength of the interference. Specifically, subjects produced |Tcom| of 182.60 ± 21.53 Nmm, 238.02 ± 21.53 Nmm, and 264.82 ± 17.56 Nmm for breaks of 10, 20, and 60 minutes, respectively. The time-dependent decay of the interference was found to have a half-life of 10.91 minutes. The 0-min break group came back after two weeks to recall the manipulation learned in context A. Subjects showed good retention of what they had learned by producing |Tcom| of 272.71± 22.91 Nmm in the first trial despite the two-week break. We propose that, when subjects are learning manipulation tasks, two components are learned in parallel: one is acquired by error-driven update of the internal model whereas the other is acquired by repetitive exposure to performing a task in the same context. The formation of the second component is context-independent and has a time-dependent inhibitory effect on the retrieval of the previously learned context-dependent first component.

Disclosures: M. Santello: None. **Q. Fu:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

Location: Halls B-H

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Program#/Poster#: 271.06/ZZ3

Topic: D.17. Voluntary Movements

Support: European Commission in Seventh Framework Programme FP7-248587

Title: Motor commands distort perception of relative fingertip position

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Abstract: Digit force modulation as a function of position for dexterous manipulation suggests that humans are skilled at sensing points of force application relative to the object and each other. However, the underlying sensorimotor transformations remain unclear. In this study we quantified subjects' ability to sense the relative position of the digits as a function of digit force without visual feedback of the hand or grasped object. Thirty subjects were asked to match perceived vertical distance between the center of pressure (CoP) of the thumb and index finger pads (d_y) of the right hand ("reference" hand) using the same hand ("test" hand). The digits of reference hand were passively placed collinearly (d_y = 0 mm). Subjects were then asked to exert normal and tangential digit forces (F*n* and F*tan*, respectively) using the reference hand, release the grasp, wait for 10 seconds, and then match the memorized d_y using the test hand. The reference hand exerted F*tan* of thumb and index finger in either same (both up or down) or opposite (thumb-index: up-down or down-up) direction. The magnitude of F*n* and F*tan* exerted

by each digit of the reference hand was the same across these four experimental conditions (4-5 N and 2.5-3.5 N, respectively). In two additional conditions, we also examined the effect of F*n* magnitude by asking subjects to exert either the same range of F*n* as for the above-mentioned conditions while exerting negligible F*tan* (4-5 N, 0 ± 0.25 N), or negligible F*n* and F*tan* (0.5-1 N, 0 ± 0.25 N). For the test hand, digit forces were either negligible (0.5-1 N, 0 ± 0.25 N; Experiment 1) or the same as those exerted by the reference hand (Experiment 2). We hypothesized that the perception of d_y would be (a) less accurate and (b) biased toward the direction of F*tan* only when the direction of F*tan* of the thumb and index finger was opposite. Subjects systematically misplaced d_y but only when digit F*tan* directions were opposite. Specifically, subjects positioned the thumb CoP higher than the index finger CoP when the F*tan* of the thumb and index finger were directed upward and downward, respectively, and vice versa ($p < 0.001$). In contrast, d_y was placed accurately when the direction of F*tan* was the same for both digits and when F*tan* magnitude was negligible. There was no difference in d_y estimation error regardless of whether the magnitude of F*n* and F*tan* of the test hand were significant or negligible. These findings indicate that the direction of F*tan* distorts the placement of the relative position of the digits. We speculate that the expected sensory consequence of motor commands (vertical separation of fingertips) overrides veridical estimation of fingertip position through actual sensory feedback.

Disclosures: D. Shibata: None. **A.M.L. Kappers:** None. **M. Santello:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 271.07/ZZ4

Topic: D.17. Voluntary Movements

Support: NSF IGERT

Title: Role of anterior intraparietal sulcus in dexterous manipulation

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Abstract: Fronto-parietal cortical networks are involved in visuomotor transformation of object properties for accurate hand shaping and sensorimotor control of digit forces. However, to date no study has investigated the neural mechanisms underlying coordination of digit position and

forces for dexterous manipulation in a natural task paradigm. When subjects perform a grasp using self-selected (unconstrained) versus predetermined (constrained) locations on an object, the trial-to-trial variability in digit position is compensated by a covariation in digit forces so as to apply appropriate compensatory torque on the object to preserve task dynamics. Manipulation with an unconstrained versus constrained grasp may require additional online monitoring and correction of digit forces in relation to where the digits make contact with the object. Here, we sought to understand the effects of temporary disruption of contralateral anterior intraparietal sulcus (aIPS) using repetitive transcranial magnetic stimulation (rTMS) on the digit position-force coordination during a constrained versus unconstrained manipulation task involving lifting an object with asymmetrical mass while preventing it from tilting. Five right-handed young subjects performed a visually-cued grasp and lift task using a precision (thumb-index) grasp at unconstrained versus constrained contacts marked on the object in separate blocks. Transient contralateral rTMS stimulation over aIPS in a given trial was either delivered prior to grasp onset, or at object contact in a random order. rTMS at either stimulation time had no effect on digit placement. However, subjects applied greater peak grip and load force rates when rTMS was delivered at object contact under both unconstrained and constrained grasp conditions ($p < 0.03$). Furthermore, rTMS to aIPS induced significant increase in preloading duration in the constrained condition when TMS was delivered at object contact ($p < 0.05$). Interestingly, we saw no disruption in the covariation between digit position and force or the compensatory torque at object lift onset. Thus, our preliminary data suggest that temporary disruption of contralateral aIPS interfered with individual grasp variables associated with digit forces, but not digit placement or digit position-force coordination. Future work will further elucidate the role of bilateral aIPS and contralateral premotor areas in dexterous manipulation.

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 271.08/ZZ5

Topic: D.17. Voluntary Movements

Support: R01 NS079664

Title: Reach-to-grasp: A single movement of the entire upper extremity

Authors: *A. G. ROUSE, A. T. ROUSSIN, M. H. SCHIEBER;
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Abstract: During reach-to-grasp, rather than reaching first and grasping second, shaping of the hand to grasp an object evolves in parallel with reaching to the object's location. Nevertheless, reaching and grasping commonly are considered to be independent processes, with the motion of proximal joints depending solely on reach location and the motion of distal joints depending solely on object shape. Here, we examined the extent to which the motion of proximal joints also depends on the object grasped and the motion of distal joints depends on reach location.
Two monkeys reached from a center location to grasp one of four objects located at one of eight radial locations separated by 22.5° as 22 joint angles were tracked. The effects of object and location on each joint's motion as a function of time were analyzed with two-way ANOVA from before the instruction/go cue until after grasp. The discriminability of object and location also was assessed with linear discriminant analysis (LDA).
The 3 shoulder joint angles showed a dominant effect of target location, but also showed a smaller effect of object that evolved in parallel. LDA using only the 3 shoulder angles discriminated not only location but also object, increasing to near 100% accuracy before object contact. In contrast, the elbow angle showed little if any effect of location (all objects were equidistant from the shoulder), but a large object effect. The 3 wrist angles showed an early effect of location which fell away as the effect of object increased. In the fingers, flexion/extension at the MCP joints showed small early effects of location followed by a larger effect of object. MCP ab/adduction angles showed later, large object effects, and the PIPs showed object effects later still. LDA using either wrist or finger angles discriminated both object and location early, but discrimination of location fell away as the time of object contact approached.
The kinematics of all upper extremity DoFs, from the shoulder to the PIP joints, thus varied substantially depending on the object to be grasped. While location affected shoulder angles most strongly, small but statistically significant differences in wrist and finger angles at different locations for a given object were observed as well. Reach-to-grasp thus is a single movement that involves both proximal and distal joints, all affected by both location and object as the movement evolves in time. Proximal joints are adjusted to achieve an attitude of the hand that permits grasping an object with a relatively invariant hand shape formed by distal joints.

Disclosures: A.G. Rouse: None. **A.T. Roussin:** None. **M.H. Schieber:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Support: R01 NS079664

Title: Reach-to-grasp: EMG activity in both proximal and distal muscles is related to both location and object

Authors: *M. H. SCHIEBER, A. T. ROUSSIN, A. G. ROUSE;
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Abstract: In reach-to-grasp movements, proximal muscles act on the shoulder and elbow to transport the hand to its target while distal muscles shape the hand to grasp the object. However, given that shoulder angles also vary depending on the object, the activity of proximal muscles might vary depending on the object as well as its location. Conversely, given that wrist and finger angles vary to orient the hand toward the object, distal muscle activity might vary with location as well as object shape.

We therefore recorded EMG activity from up to 16 muscles in the arm and hand of two monkeys performing reach-to-grasp movements involving four objects in up to eight radial locations separated by 22.5°. In general, EMG activity occurred in two temporal epochs. A first burst of activity occurred around the onset of movement. After this initial burst, the overall level of EMG activity decreased. A second increase in EMG activity began shortly before and continued through object contact. In the initial epoch, EMG activity appeared predominately in proximal muscles, wrist extensors and extrinsic digit extensors. In the second epoch, proximal muscles along with wrist and extrinsic digit flexors were more active. Also during this second epoch, the intrinsic muscles of the hand became active, and their activity was maintained during contact with the peripheral object.

In proximal arm muscles, EMG activity during the first epoch varied predominantly with the location to which the subject reached, although some proximal muscles also showed some object-related activity. In the second epoch, surprisingly, proximal muscle activity varied primarily depending on the object being grasped. The wrist and extrinsic digit extensors' activity during the first epoch depended on both object and location. In contrast, wrist and extrinsic digit flexors became active in the second epoch with their activity being affected more by object than location. Finally, intrinsic muscles of the hand showed predominately object-related activity during the second epoch.

For the present reach-to-grasp movements, an initial epoch of EMG activity in both proximal and distal muscles appeared to transport and orient the hand toward the target object. A second epoch of EMG activity--comprised mostly of object-related variation not only in distal, but also in proximal muscles--positioned proximal joints to achieve the appropriate attitude of the hand and closed the hand on the object. Although occurring in differing proportions, in each epoch the activity of both proximal and distal muscles varied significantly depending on both the reach location and the object grasped.

Disclosures: M.H. Schieber: None. **A.G. Rouse:** None. **A.T. Roussin:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Title: Pre-tensioning of musculotendons is necessary to achieve finger postures and slow finger motions

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Abstract: While the mechanics and neural control of rapid limb movements is relatively well understood, it is currently unknown how individual muscles are controlled to produce slow finger motions. Our purpose is to identify the necessary conditions for which the dominant models of muscles as elastic force actuators [1] can hold an arbitrary posture in the finger workspace--and produce quasi-static finger motions across them. Our test system is a 2-joint, 4-muscle planar finger model. Our approach is to define the set of muscle stiffness parameters that make it feasible to hold specific postures and transition among them [2] [3]. That is, we identify whether or not muscle stiffness parameters exist to produce a strain (i.e., potential) energy minimum for every finger posture in the workspace. First we explore the combinations of muscle stiffnesses that can keep the finger in equilibrium across its workspace. We find that such biomechanical systems have no solutions if muscles are not "pre-stretched," i.e., that muscles must always have strain energy, even at their shortest lengths. By adding a fixed initial stretch to each of the muscles and thus making them pre-stretched, we observe the feasibility of holding any posture in the workspace, and smoothly transitioning across postures by simply tuning muscle stiffness. We then solve the inverse problem where we identify the range of stiffness values across muscles to hold every posture in the workspace, which also allows us to identify the most energetically efficient muscle activations. We find that many postures in the workspace can be achieved by a variety of values and combination of muscle stiffnesses, but other portions

of the workspace require very precise values and combination of muscle stiffnesses. These novel results begin to explain the types of neural strategies necessary for slow finger motions, and begin to provide a neuromechanical explanation for a variety of functional deficits and deformities seen in neurological disorders.

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[1] Zajac, F. E., Muscle and tendon: properties, models, scaling, and application to biomechanics and motor control. Critical reviews in biomedical engineering, 1989. 17(4): p. 359-411.
[2] Valero-Cuevas, F.J., A mathematical approach to the mechanical capabilities of limbs and fingers. Progress in Motor Control, 2009: p. 619-633.
[3] Valero-Cuevas, F.J., et al., Computational models for neuromuscular function. Biomedical Engineering, IEEE Reviews in, 2009. 2: p. 110-135.

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Program#/Poster#: 271.11/ZZ8

Topic: D.17. Voluntary Movements

Support: CIHR

Title: Compensating for discrete contact forces through grip force modulation and choice of contact location

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Abstract: Many day to day actions involve the manipulation of objects. During basic manipulation tasks_such as when an object is held and moved around_we see that grip force (i.e., the normal force applied to the object surface by the contacting digits) modulates in synchrony with the acceleration-induced load forces resulting from object motion. Often times however, a discrete load may be superimposed on the basic manipulation task, such as when using a hand-held object to contact a second object. In this context, a person must accommodate these sudden changes in load force, induced by the contact event, through compensatory changes in grip force applied to the hand-held object. Indeed, these grip force adjustments must be scaled to the dynamics of the contacted object, which may render some contact locations preferable over others. We tested this presumption using a novel task that involved moving a hand-held object to

contact a bar that pivoted counterclockwise about a fulcrum. Participants held an object in a precision grip and moved it in the horizontal plane to contact the bar with sufficient force to cause it to rotate 90° from its resting orientation that was orthogonal to the long axis of movement. As a consequence of the dynamics of the pivoting bar, the force necessary to achieve this rotation decreased as a linear function of contact distance from the pivot. Participants were instructed to contact the bar at a location that felt comfortable and natural. Two conditions were performed in which the centre of the bar was shifted either left or right of the midline, positioning the fulcrum either more distant or closer to the midline. Preliminary results from these conditions suggest that participants bias their reach location to a position on the bar that required less contact force to rotate it. That is, participants biased their endpoint to a location on the bar away from the fulcrum. Notably, participants did not simply reach for the position most distant to the fulcrum on each movement. This finding may indicate that participants tradeoff the impact force required for rotation and movement distance in choosing their contact location. Preliminary evidence also suggests that participants modulate their grip force to account for the position dependent changes in contact force along the bar. Furthermore, these changes are evident just prior to the contact event indicating that participants predictively modulate their grip force to account for the discrete loads.

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Support: Prodex

ESA

PAI

FNRS

ARC (Belgium)

Title: Motor control of the arm during voluntary collisions in inverted gravity field

Authors: *V. THEATE[1], C. PONSELET[2], T. JEAN-LOUIS[3], L. PHILIPPE[2];
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Catholique De Louvain, Louvain-La-Neuve, Belgium; [3]IONS, Univ. Catholique De Louvain, Brussels, Belgium

Abstract: Predictable collisions during object grasping are common in everyday life and previous studies have reported asymmetries between up and down collisions in the control of precision grip (White et al., 2011 & 2012). The aim of this study is to investigate the relative role of movement direction (to the head or to the feet) versus gravity (+1g versus -1g) in these asymmetries.
In our experiment, six subjects were seated and restrained in a chair. They performed collisions in direction of their head (H) or in direction of their feet (F), randomly interleaved. These collisions were, first, performed in a normal gravity configuration (+1g) and, second, in an inverted gravity configuration (-1g). The latter consisted in turning the subject with the head upside down. We recorded 300 collisions by subject for each of these 4 categories (+1gH, +1gF, -1gH, -1gF). Dynamics of precision grip, movement kinematics and muscular activities were recorded.
Our results show that the patterns of muscular activities adapt immediately to the gravity configuration (+1g versus -1g). Very interestingly, the kinematics of the upper limb is the same with respect to the allocentric frame of reference in both configurations (+1gH versus -1gF and +1gF versus -1gH). As a consequence, the load force at peak acceleration depends on the movement direction with respect to gravity, whatever the direction of the movement with respect to an egocentric frame of reference (to the head versus to the feet). Thus the load force at peak acceleration is the same when the movement is opposite to gravity (+1gH and -1gF) and is much larger than when the movement is in the direction of gravity (+1gF and -1gH). In contrast with this adaptation of movement kinematics, the dynamics of the precision grip does neither fully adapt during the transport phase (at peak acceleration) nor at collision (impact with target)

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Support: JSPS KAKENHI Grant number 23500675

Title: Prehension force for lifting a light-weight object

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Abstract: Ability to manipulate small objects at the fingertips is an indispensable sensory-motor function in our daily lives. Previous studies of prehension force control commonly used a test object weighing greater than 100 g. In the present study, an attempt was made to develop an extremely light (6 g) test apparatus equipped with grip and lifting (load) force sensors. Using this apparatus and healthy young adults (N = 20), we examined grip-load force coordination in a light weight range. The subjects lifted the apparatus using the thumb and index finger, held it in the air for 10 sec, and drops it by slowly separating the fingers. Rayon and sandpaper grip surface, and weights of 6 g - 200 g were tested. Static grip force, slip force, a static grip force/load force ratio, safety margin relative to the static force, and the coefficient of static friction were evaluated. In both surfaces, the static grip force/load force ratio increased non-linearly with weight lighter than 30 g. The coefficient of friction also had a similar trend. The relative safety margin was quite large (> 80%) for the 6 g apparatus with both surfaces, which decreased non-linearly with load force, and reached about a half value while lifting the 200-g apparatus. When the subjects were asked to grip with smaller static grip force than usual force, static grip force was decreased by 40% on average due to a decreased safety margin. However, even with this controlled small static grip force, the higher relative safety margin during light object lifts remained. The findings suggest that force coordination for lifting of light objects (< 30 g) differs from that for heavier objects. Measurement of the area of finger-surface contact, and skin deformation in relation to grip force suggested that the high relative safety margin during manipulating light objects could be a strategic behavior to compensate decreased cutaneous input from the skin.

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Support: Grant-in-Aid for Scientific Research (C) No. 08042773 from the Japan Society for the Promotion of Science

Research grant from Niigata University of Health and Welfare

Title: Gaiting of cutaneo-muscular reflexes and somatosensory evoked potentials differs with the load compliance during maintaining constant finger force or position

Authors: *H. KIRIMOTO, T. MATSUMOTO, H. TAMAKI, M. SUZUKI, S. MIYAGUCHI, K. SUGAWARA, H. ONISHI;
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Abstract: Introduction; When an individual performs a submaximal statistic contraction, there are two different load types either to produce a constant force by pulling against a noncompliant restraint (force task) or to maintain a constant limb angle while supporting an equivalent isoinertial load (position task) . Sensorimotor modulation is crucial for executing smooth voluntary movements in humans, and many studies have examined this by using attenuation of cutaneo-muscular reflexes (CMR) and somatosensory evoked potentials (SEPs) during or even before the initiation of voluntary movement, which is known as 'gating'
Objective; The purpose of the current study was to determine the influence of load compliance during muscle static contraction tasks on the gating effect, i.e., attenuation of CMR and SEPs during the motor task.
Methods; Fourteen healthy subjects contracted the right first dorsal interosseus muscle by abducting their index finger 50 seconds either to produce a constant force against a rigid restraint by 10 degree (force task) or to maintain a constant position against a constant load of 20% of maximum contraction (position task). CMR and SEPs were recorded, following digital nerve stimulation of the index finger while subjects kept contraction. CMR were recorded from the first dorsal interosseous muscle and SEPs were recorded from C3' (2 cm posterior to C3). The stimulus was delivered using a constant current stimulator at a level 2.5 times above that required for perception (pulse duration 100 ms, frequency 5 Hz for CMR / 2Hz for SEPs). The amplified and filtered EMG and EEG signal were averaged time-locked to the stimulus for 250 sweeps
Results; The E2 component of the CMR and the P14/N20 components of the SEPs were significantly reduced during position task compared with force task ($p<0.001$). There was a significant (qualitative) relationship between the decrease in the size of the E2 component of the CMR and the P14/N20 components of the SEP (X2 test; $P < 0.05$).
Conclusions; We conclude that the decrease in size of the E2 component associated with position task results from gating of the digital nerve input. Larger gating effect in the position task could imply that the task to maintain the position of the index finger while supporting a constant load requires more afferent information with which the enhanced gating of centripetal mechanism would occur.

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Support: NSF (IIS/HCC) 0964220

Title: Finger and arm control during interactions with multitouch devices

Authors: *D. L. JINDRICH[1], D. S. ASAKAWA[2], J.-H. LEE[3], C. LOZANO[4], J. T. DENNERLEIN[5];
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Abstract: Touchscreen and gestural computing devices, including tablets and smartphones, are commonly used for both personal and work purposes. These rapidly spreading devices involve complex finger and thumb movements whose motor control is poorly-understood. We are using experimental and modeling studies to better understand the control of multitouch interaction. We measured finger joint kinematics and arm muscle EMG during common one- and two-finger gestures used on a hand-held touchscreen computing device. Two-finger gestures involved larger changes in joint angles of the digits and greater muscle activity than one-finger gestures. Muscle activity was substantial, of magnitudes associated with risks for musculoskeletal disorders. Holding the device was associated with increased EMG relative to interacting with a device supported on a surface. Surprisingly, turning the device on and providing contextual feedback resulted in decreased EMG relative to interacting with a device that was turned off. To better understand the specific neuromuscular control strategies associated with multitouch gestures, we are using detailed musculoskeletal models of the hand and arm. We have incorporated intrinsic hand muscles into an existing musculoskeletal model of the arm using techniques for data-driven optimization to determine attachments. Optimization methods can reproduce measured moment arms for intrinsic and extrinsic muscles and tendons of the index finger. Our combined experimental and modeling approach will contribute to understanding the motor control of complex gestures, designing interfaces that increase performance, reducing risks for musculoskeletal disorders, and improving accessibility to motor-impaired populations.

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Title: Does abrupt inversion of visual feedback influence grip force adjustments during object transportation? Implication for forward models

Authors: *F. DANION[1], F. SARLEGNA[2];
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Abstract: It has been proposed that goal-directed movements are executed by means of a central controller, an inverse model which can convert
desired hand or object motion into adequate motor commands (Wolpert and Kawato, 1998), in conjunction with a predictor (forward model) that can estimate the sensory consequences of the movement (Desmurget and Grafton 2000). Forward models have been hypothesized to be useful in the context of visuomotor tasks (Miall et al. 1993; Mehta and Schaal 2002) and object manipulation tasks (Flanagan et al. 2003; Danion and Sarlegna 2007), as both would allow controlling the motion of the object and adjusting grip force adequately. The goal of the current study was to investigate how independent are these two types of forward model, by means of a task requiring adaptation of visuo-manual tracking but no adaptation of grip force control. Thirteen healthy adults tracked a randomly-moving target by moving a handheld object that was restrained by an elastic cord. The set-up is similar to that used in Sarlegna, Baud-Bovy and Danion (2010). The ongoing position of the object was displayed as a cursor on a screen along with the visual target. The ability to manipulate the cursor/object was monitored through the accuracy of visuo-manual tracking and the coupling between the grip force and the load force resulting from the tension of the elastic cord. As expected, the sudden inversion of the Relationship between the hand displacement and the cursor displacement resulted in lower tracking performance. However, as participants had more and more experience with the sudden inversion, tracking performance increased, suggesting that the related forward model was updated. Surprisingly, at no point during the training course was the predictive control of grip force affected by the visuo-motor inversion. Overall, these results suggest that the forward model underlying visuo-manual tracking can be updated independently of the forward model underlying the predictive control of grip force.

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Poster

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Topic: D.17. Voluntary Movements

Title: Neural representation of an invariant object mass is spatiotemporally changing during object manipulation

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Abstract: We need to estimate the properties of objects when we interacts with the external world. This kind of estimation is especially important when we manually manipulating objects. For example, appropriate estimate of inertial properties of a hand-held coffee cup is essential for successful and efficient lifting and transporting it to our mouth. A wrong estimate of mass distribution will tilt the cup and spill the coffee; an overestimation of its mass will incur excessive force on the cup. How the nervous system represents the object estimate has not been systematically investigated.
We address this question with a novel motor perturbation paradigm. We asked subjects to perform center-out hand reaching task while holding a water-filled cup. After several attempts subjects learn the cup mass as such the movement trajectory becomes straight in 3D space. However, when the cup is unexpectedly emptied or filled in between trials, subjects will make vertically-curved reaches. This height change can be quantified as a surrogate of subjects' estimates about the object mass (Yan et al., 2013). In Experiment 1, we find that the mass estimate, learned in one reaching direction, can be generalized to other directions. Surprisingly, despite of repetitively lifting constant mass, this spatial generalization is not full and uniform across directions: the more deviated from the learning direction, the less generalization can be seen. This Gaussian-shaped generalization function is similar to what have been shown in other motor generalization tasks involving complex dynamic perturbations. In Experiment 2, when the test trials for probing the mass estimate are performed 30 or 60 seconds after the training trials, the mass estimate is significantly altered as if the sensorimotor memory of the object mass decays. In Experiment 3, when subjects explicitly observe (and verbally inform about) the change of object mass, they still cannot fully generalize the learning from one direction to other directions.
Our results indicate that when the inertial property of an object is learned via movements its correct representation is only available for subsequent actions performed near the learning

direction and within a short timing window. Verbal knowledge or visual observation is not sufficient for establishing correct representation of object inertial properties. It thus suggests that while the high-level cognitive representations of an object are spatially invariant and temporally sustainable, the neural representations of movement-related object properties might have their unique spatiotemporal patterns.

Disclosures: G. Kong: None. **K.L. Wei:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: D.17. Voluntary Movements

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Title: Physiologically-based control of a robotic tendon-driven system

Authors: *J. ROCAMORA[1], C. M. NIU[1], J. BUCHLI[2], T. D. SANGER[1], F. J. VALERO CUEVAS[1];
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Abstract: We have proposed a synthetic analysis approach to validate our models and hypotheses of neural control. We focus on tendon-driven systems to experimentally replicate the neuromechanical function of the fingers. However, such systems are generally high-dimensional and nonlinear, which require a careful strategy for their control.
In previous work [1], we explored theoretical control strategies for these systems by developing the first stochastic optimal controller applied to a simulated index finger model. Recent work [2] led to the actual implementation of such an approach in a tendon driven robotic finger.
We present the extension of our modeling work by including additional physiological elements such as generic muscle excitation-contraction dynamics and muscle spindle models [3]. To control this expanded system, we use the Reinforcement Learning approach PI2-CMA algorithm

(Policy Improvement with Path integrals with Covariance Matrix Adaptation) as described in [4] that allows more autonomous tuning of control and learning parameters, resulting in better convergence. This non-physiological machine learning approach identifies the constraints and boundary conditions for future neuromorphic control, which incorporates spiking neurons and realistic neural circuitries. It eventually allows us to test which minimalist structures are needed to achieve dexterous hand control, as well as the origins of upper-limb neuropathology.
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[1] E. Theodorou, E. Todorov and F. J. Valero-Cuevas. Neuromuscular Stochastic Optimal Control of a Tendon Driven Index Finger Model. American Control Conference, 2011.
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Disclosures: J. Rocamora: None. **C.M. Niu:** None. **J. Buchli:** None. **T.D. Sanger:** None. **F.J. Valero Cuevas:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Support: AHA Grant 13PRE14610017

Title: Kinematic and reflexive finger-thumb coupling during pinch

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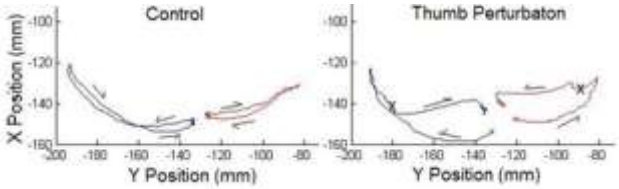
Abstract: The ability to precisely and dynamically coordinate activity of the thumb and fingers is fundamental to our development as humans. Use of day-to-day objects such as scissors or pens requires dynamic coordination between the digits. Unfortunately, this critical coordination

between the fingers and thumb is significantly impaired following stroke. The nature of these deficits, however, is not yet well understood due to the difficulty of precisely measuring and acting on the hand under dynamic conditions. In this study, we employ a high bandwidth, precise finger exoskeleton to deliver perturbations to the index finger during pinching movements of the finger and thumb. The exoskeleton provides for full control of either torque or angle at each flexion/extension joint of the index finger. Servomotors located proximally to the hand transmit torque through a set of cables to the joints of the exoskeleton which are aligned with those of the finger. Both torque and reflex-inducing perturbations are applied in order to analyze the extent of kinematic and reflexive finger-thumb coupling involved in motor control of pinch. Preliminary results show that in slower movements (10s) when a perturbation is delivered to one digit with no outside interference in the other, a corresponding pause arises in the trajectory of the unperturbed digit (Fig. 1). Thus, substantial non-reflexive coupling between the index finger and thumb was apparent during movement. Knowledge of this coupling will inform future rehabilitation strategies and may be utilized to reduce the cost of take-home assistive and therapeutic devices.

Fig. 1 - Example of finger-thumb endpoint path (left-right traces) during unperturbed pinch (left) and including perturbation of the thumb during close (right). Onset of perturbation is marked with an ‘X’ and direction of motion is indicated by arrows.



Disclosures: C.L. Jones: None. **D.G. Kamper:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 271.20/ZZ17

Topic: D.17. Voluntary Movements

Title: The effects of gender and hand strength differences on the relative contribution of efference copy and sensory feedback to the sense of effort

Authors: *D. E. ADAMO[1], S. SCOTLAND[2], B. J. MARTIN[2];
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Abstract: Considering asymmetry in the sense of effort, a possible peripheral modulation of the sense of effort, and gender dependence of sensory asymmetry; the aim of this study was to investigate the effects of gender and hand strength differences between the dominant and non-dominant hand on the relative contribution of efference copy and sensory feedback to the sense of effort. Thirty-two (22 F and 10 M) right-handed, healthy adults performed grasp force matching tasks while seated in a standard posture with their forearms supported. Individuals were grouped according to grip strength differences between dominant and non-dominant hands, as either; positive (dominant at least 5% stronger than non-dominant), equal (dominant - non-dominant grip strength less than 5%), and negative (non-dominant at least 5% stronger than dominant). Participants reproduced a right or left hand 20% MVC reference grasp force with the same (ipsilateral remembered, IR) or opposite (contralateral remembered, CR) hand, with or without reference force visual feedback and, with or without reference hand vibration (60Hz) applied to flexor tendons at wrist level. A marker controlled by the reference force signal and presented on a vertical scale was displayed on a video screen when visual feedback was permitted. In the absence of visual feedback the reference force was learned from verbal qualitative and quantitative feedback. Feedback was never provided for the matching hand. Since vibration alters sensory coding of ongoing motor activities and muscle tension, it was hypothesized that vibration-induced changes in sensory information would influence the magnitude of force matching errors that, further, would be influenced by gender, grip strength differences between hands and the availability of visual feedback. The results revealed inconsistencies in the matching constant error expressed in both % MVC and Newtons. Variations in matching asymmetries found for different conditions suggest that the contribution of efference copy and sensory feedback to the sense of effort reflect complex interactions between gender and hand strength differences. The findings further emphasize the context dependent and multifaceted nature of grasp force production and control.

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Program#/Poster#: 271.21/ZZ18

Topic: D.17. Voluntary Movements

Title: Temporal aspects of grasping apertures with different tools

Authors: *Y. ITAGUCHI, C. YAMADA, K. FUKUZAWA;
Waseda University, Psychology Dept., Shinjuku-Ku Tokyo, Japan

Abstract: The present study investigated motor control of hand aperture in the process of grasping an object. Previous studies have suggested that an effecter-independent motor representation is used in grasping an object. Finger pre-shaping aperture was equally scaled as a function of object size. Temporal aspects of the grasping movements were, however, different between hand and tool use. To further elucidate how a grasping movement is affected by different effectors, the present study examined
the temporal transitions of grasping aperture using two different tools.
Methods
Five participants reached and grasped an object with (1) a hand (thumb and index finger), (2) chopsticks, and (3) a scissors-like tool. Diameters of the target object was 1.5cm, 2cm, and 2.4cm, with 2cm in height. The object and a start position of the movements were located a 20cm distance away on the midline of the participants. The start point was located at a point 25cm from the participants. The participants carried out a total of 180 trials (20 trials × 3 objects × 3 grasping conditions). The order of the objects grasped was randomized among the participants.
Results
(1) Maximal grip aperture (MGA) was equally scaled to object size in the three grasping conditions, i.e., the size of MGA increased as the object size increased. The size of MGA in the hand condition was larger than in the two tool-use conditions. (2) Timing of the MGA did not vary among the three conditions; MGA appeared at about 75% of the movement time. (3) When participants used tools, a grip aperture plateau was followed by MGA, and its duration was most prolonged in the scissors-like tool condition; 90% or more of MGA was maintained for 25%, 17%, and 13% of the movement time before the MGA in the scissors-like tool, chopsticks, and hand onditions.
Discussion
The present study provided mainly two new findings. First, sizes of MGA were modulated equally by object size, but the sizes of MGA were not the same among the grasping effecters. This finding suggests that absolute size of MGA was not a critical index in the motor planning processes. Second, a plateau in the temporal transition of grasping aperture was evident in the tool use conditions *before* the timing of MGA. This result was partially inconsistent with a previous study (Gentilucci et al. 2004) where a plateau *followed* MGA. This apparent disagreement may be attributed to the mechanical characteristics of the tools. In sum, the present results showed what aspects are effecter-independent and effecter-dependent in the temporal transition of grasping aperture.

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Poster

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Topic: D.17. Voluntary Movements

Title: Psychophysical limitations and biases in the perception of tactile direction

Authors: *J. C. TANNER[1], N. NEWMAN[2], N. ZAUTRA[3], S. HELMS TILLERY[1];
[1]SBHSE, [2]CLAS, [3]SOLS, Arizona State Univ., Tempe, AZ

Abstract: Some of the tactile cues which arise from our interactions with objects have an element of directionality. These cues can inform grasp intent, adjustment, and in some cases may even modulate a response. In order to quantify perceptual limits of tactile direction, we investigated the perception of two types of tactile direction: oriented edges pressed passively into the skin and ulno-radially directed slip during precision grip. In both experiments, subjects are presented a reference stimulus and then a test stimulus at a randomized angle at 5° increments up to 30° relative to the reference. For the oriented edges, 8 subjects placed their right hand in a device that held the index finger over the stimulus bar. Subjects received a 1 second reference stimulus of 0°, parallel to the subject, or 90°, perpendicular to the subject, followed with the 1 second test stimulus after a randomized time. For the slip experiment, 4 subjects placed their arm in a mount that allowed for digital movement, but restrained the wrist to eliminate induced wrist, elbow and shoulder movement. Movement of each joint was monitored via PhaseSpace motion capture. The subject was presented with a 2”x2” textured object and instructed to use two grips: loaded, as if to control the object, and unloaded, as if the object were slipping. The object was translated 1 cm in 0° (proximal), 90° (up), 180° (distal), and 270° (down). This reference stimulus was followed by a 1 cm test stimulus at the same angular increments as in the previous experiment. A bias for distal and downward movements is hypothesized as physiological experiments exhibit sensitivity to those directions, shown via lower latency reaction times and higher force grip responses. After each pair of stimuli, subjects responded “Same” or “Different,” indicating if they perceived the experimental stimuli to be a repeat of or deviation from the reference. We found that the threshold for detecting a change in the orientation of the oriented bar was at approximately -15° and 20°. There is a slightly lower threshold for perpendicular stimuli (-20° and 20°) versus the horizontal stimuli (-20° and 25°). Presently, there is no significant difference between subjects’ perception of change in the stimulus for the second experiment. The data demonstrates a reverse bell curvefor the psychometric curve (% Response Different vs Angular increment), but the variation in data is high. Preliminary experiments that used larger angular increments and only one grip type indicated a threshold of 20° and a bias in the vertical directions. However, the renovated experiment requires more subjects as n=3 is inadequate for a sample population.

Disclosures: J.C. Tanner: None. **N. Newman:** None. **N. Zautra:** None. **S. Helms Tillery:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Title: Effects of force production and working memory on pain perception

Authors: *S. COOMBES, T. PARIS, G. MISRA, D. B. ARCHER;
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Abstract: The goal in the current study was to examine the analgesic effects of a pinch grip-force production task and a working memory task when pain-eliciting thermal stimulation was delivered simultaneously to the left or right hand during task performance. Control conditions for visual distraction and thermal stimulation were included, and force performance measures and working memory performance measures were collected and analyzed. Our experiments revealed three novel findings. First, we showed that accurate isometric force contractions elicit an analgesic effect. Second, the magnitude of the analgesic effect was not different when the pain-eliciting stimulus was delivered to the left and right hand during the force task or the working memory task. Third, we found no correlation between analgesia scores during the force task and the working memory task. Our findings have clinical implications because they show that acute force production by one hand has an analgesic effect on pain that is simultaneously experienced in the other hand. From a theoretical perspective we interpret our findings on force and memory driven analgesia in the context of a centralized pain inhibitory response.

Disclosures: S. Coombes: None. **T. Paris:** None. **G. Misra:** None. **D.B. Archer:** None.

Poster

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01NS027484

Title: Sequence-dependent hand kinematics by professional and amateur pianists

Authors: *S. A. WINGES[1], S. FURUYA[2];
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Abstract: Long-term musical training enables dexterous finger movements. Behavioral studies using the individuated finger movements demonstrated that trained pianists move fingers more independently than the untrained individuals. By contrast, studies using a transcranial magnetic stimulation revealed loss of surround inhibition of the finger muscles, which suggested a larger covariation of movements across fingers for more skilled players. To address this issue, the present study aimed to describe skilled finger movements during musical performance by professional and amateur pianists. Five professional pianists and five amateur pianists were asked to play thirteen musical pieces for ten successive trials at a certain tempo (inter-keystroke interval = 125 ms). Timing and force of each keystroke were recoded as MIDI information with a time resolution of 2 ms, and dynamic hand posture was recorded using sensors embedded in a right-handed glove (CyberGlove) with a time resolution of 12 ms. In order to assess effects of fingering on the finger movements, our analysis focused on three successive keystrokes, in which the central keystroke was with each of the five digits. The results of MIDI data showed that spatiotemporal features of the keystrokes were not independent of fingering. For example, during a keystroke with the thumb, the finger-key contact duration was shorter when the preceding finger was the little finger than the index finger for both of the two groups ($p<0.01$). In addition, the variability of rhythm and force of the keystrokes was overall larger for the amateurs than the professionals, which confirmed finer control of finger movements in more skilled players. The kinematic information also varied in relation to fingering and group at some joints. Of particular interest is that a group difference in the correlation coefficients of the movements between two fingers was dependent on the fingering, which indicates that the professionals moved the fingers more and less independently than the amateurs depending on the fingering. It is therefore likely that skilled pianists flexibly change the finger joint coordination across movement sequences.

Disclosures: S.A. Winges: None. **S. Furuya:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Support: University of Houston Small Grants Program

Title: Effects of task complexity on grip force production

Authors: *A. N. KHAN, S. L. GORNIAK;
Hlth. and Human Peformance, Univ. of Houston, Houston, TX

Abstract: Previous studies of fine motor control have focused on the ability of participants to match their grip force production to a visually provided template. Widespread use of this technique ranges from studies of healthy control participants to evaluation of interventions for movement disorders. Typical visual templates have included both constant and variable force production values (eg. sine waves). The use of variable force production templates has been viewed as providing a challenging motor task in comparison to constant force production; however, the complexity/difficulty of these tasks has not been evaluated to date. In the current study, we investigated the behavioral differences exhibited in fine grip force control during several variable force production templates. Our results indicate that behavioral differences exhibited in variable force production tasks can be detected by using non-linear analyses. Use of these non-linear techniques allows for detection of behavioral differences reflecting task differences in non-Fitts-type tasks.

Disclosures: A.N. Khan: None. **S.L. Gorniak:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Support: German Ministry for Education and Research (BMBF, 01GQ1102)

Title: The functional topology of large-scale brain networks relates to the efficiency of short-term skill acquisition training

Authors: *Z. ZANG[1], L. GEIGER[1], M. ZANGL[1], A. SCHAEFER[1], H. CAO[1], J. REIS[3], M. RUF[2], A. MEYER-LINDENBERG[1], H. TOST[1];
[1]Dept. of Psychiatry and Psychotherapy, [2]Dept. Neuroimaging, Central Inst. of Mental Health, Heidelberg Univ., Mannheim, Germany; [3]Dept. of Neurol., Albert-Ludwigs-University, Freiburg, Germany

Abstract: The acquisition of complex motor skills involves rapid plasticity-related reorganization of large-scale brain functional networks linked to movement control, working memory, and spatial attention, but the relationship to brain functional network properties derived from graph theory methods is largely unexplored. Here, we examined the relationship between the efficiency of skill acquisition training and the brain functional topology during resting state in 54 healthy volunteers (mean age 27.1 ± 7.9 years, 27 males). Prior to neuroimaging, motor skill learning was induced in a single training session involving 30 minutes repeated practice of a sequential visual isometric pinch task, an established behavioral marker for healthy and disturbed motor skill learning linked to cortical-striatal function and the effects of plasticity genes (Fritsch et al., 2010). Skill acquisition was defined as practice-induced changes in the speed-accuracy tradeoff function over time, which accounts for training-induced variations in sequence errors and movement speed (Reis et al., 2009). Resting state fMRI data were acquired in a 5 minute scan at 3T. Mean time series from 264 functional regions of interest (Power et al., 2011) were extracted after regressing out head motion effects, white matter and cerebral spinal fluid signals. Pairwise temporal correlations matrices were calculated and thresholded at in 10% intervals over a range of 5 densities (10% - 50%). Connectivity and graph properties were calculated using the Brain Connectivity Toolbox (Rubinov & Sporns, 2010). Partial correlation analysis of Global network properties (i.e., smallworldness, global efficiency, modularity, mean path length) with skill increase over training was performed while controlling for the effects of age and sex. The analysis of the behavioral data confirmed a significant training-induced increase in skill over time ($p = 4.7 \times 10\text{-}12$). Smallworldness, global efficiency, and modularity were significantly positively correlated with individual short-term skill acquisition abilities across all examined densities (range of r's = 0.28 to 0.47, range of p's = 0.041 to 0.003). Moreover, mean path length showed a significant negative correlation with skill acquisition across densities (range of r's = -0.338 to -0.484, p's = 0.014 to 0.0003). These data demonstrate the value of fMRI-based graph theory methods for the exploration of the neural correlates of learning and plasticity and suggest that individuals with more integrated, efficient and economic brain functional topologies are better equipped to learn complex motor skills in a short amount of time.

Disclosures: Z. Zang: None. **L. Geiger:** None. **M. Zangl:** None. **A. Schaefer:** None. **H. Cao:** None. **J. Reis:** None. **M. Ruf:** None. **A. Meyer-Lindenberg:** None. **H. Tost:** None.

Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Title: Decoding grasp-relevant dimension of 3D objects in the Lateral Occipital Complex in grasping and viewing tasks

Authors: *S. MONACO[1], R. KAMRAN-DISFANI[2], K. FIEHLER[3], D. Y. HENRIQUES[1]; [1]Ctr. for Vision Res., [2]York Univ., Toronto, ON, Canada; [3]Justus-Liebig Univ. Giessen, Giessen, Germany

Abstract: The lateral occipital complex (LOC) in the ventral visual stream is known to be involved in visual object recognition. Despite the well-established role of ventral visual stream areas in object representation, growing evidence highlights the contribution of these areas to action planning and execution (Gallivan et al. 2013). However, extent to which LOC encodes overall object properties (e.g. size) as opposed to action-relevant object information (e.g. grasp-relevant dimension) is still not fully understood. The aim of this study was to determine which object properties could be predicted by LOC activity when an object was grasped or passively viewed.

We addressed this question by using a slow-event related fMRI design. We manipulated size (S) and grasp-relevant dimension (D) of 3-dimensional (3D) objects in grasping and viewing tasks. The grasp-relevant dimension of each object coincided with the top-to-bottom axis of the object, according to which the grip had to be scaled. The objects were shaped as a square (small S, small D) and a rectangle presented vertically (large S, large D) or horizontally (large S, small D). The task (grasp, view), object grasp-relevant dimension (large, small), and object size (large, small) varied in successive trials, yielding a 2 x 2 x 2 factorial design.

Fourteen right-handed volunteers participated in this study. At the beginning of each trial, an auditory cue instructed participants about the task. After 2-s, an object was illuminated for 250-ms, cuing the participant to initiate the task. For the purpose of previous published analyses exploiting repetition suppression (Monaco et al, 2013), a second object was presented 4-s after the first one and was followed by a 16-s ITI. However, for the present study only the first object presentation was included in the analysis. Multivoxel pattern analysis was performed using a support vector machine binary classifier.

Results from five participants show that the activity pattern in LOC during grasping could be used to decode between large and small grasp-relevant dimension, regardless of whether object size differed. Moreover, the activity pattern in viewing conditions could be used to decode between objects only when both size and grasp-relevant dimension differed, but not when either one was the same. These results show that object dimensions that are critical for the execution of accurate grasping movements can be predicted by LOC activity. In addition, the different decoding abilities in viewing and grasping conditions suggest that the results for grasping are not merely an epiphenomenon of the visual stimulation, but rather a product of information processing specific to action.

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Poster

271. **Voluntary Motor Control: Finger and Grasp**

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Topic: D.17. Voluntary Movements

Title: Wrist forces and torques during activities of daily living

Authors: *A. L. PANDO[1], J. N. HERNANDEZ[2], S. K. CHARLES[3];
[1]Mechanical Engin., [2]Neurosci., [3]Mechanical Engin. and Neurosci., Brigham Young Univ., Provo, UT

Abstract: The wrist is one of the most common sites of joint injury, sustaining two-thirds of injuries due to repetitive motion. Although excessive force/torque is thought to be the leading cause of injury, the forces and torques that the wrist encounters in everyday life are virtually unknown. The purpose of this study is to characterize the forces and torques encountered in normal, healthy wrist activity during activities of daily living (ADL). The findings will enhance our knowledge of natural wrist movement, normal ranges of wrist activity, and how to properly rehabilitate for ADL.

Eleven young, healthy subjects (5 male) participated in this study. Electromyographic (EMG) sensors recorded activity of the four primary wrist muscles. Subjects performed 25 ADL (5 repetitions apiece). The following measures were computed: muscle force, joint torque, and co-contraction. Muscle force was calculated from the experimental EMG data and a position-dependent proportionality constant (relating EMG to force). Analysis considered all ADL cumulatively and involved comparing muscular contraction levels, forces exerted by each muscle, torque magnitudes and directions, and co-contraction activity over various torque and contraction levels.

Each muscle exerted differing levels of activation compared to their maximum voluntary contraction (MVC). The extensors, on average, exerted more of their strength than the flexors (13% and 6% of MVC respectively). The most frequent level of contraction occurred at 5% of MVC. The average amount of force produced by each muscle was (in increasing order) 65 N by the extensor carpi ulnaris, 70 N by each flexor, and 125 N for the extensor carpi radialis brevis-longus complex. The average amount of torque exerted by the wrist was 2 Nm, and the most frequent amount was 0.3 Nm (maximum wrist torque is on the order of 12 Nm). More torque was exerted in radial-ulnar deviation (RUD) than in flexion-extension (FE) for all muscles except the flexor carpi radialis.

Frequent co-contraction occurred over all levels of contraction. The frequency of co-contraction

was directly proportional to muscle activation, except in RUD during moderate-to-high levels of torque (more than 1.8 Nm), where the frequency of co-contraction was similar for all contraction levels greater than 40% of MVC.
These findings characterize natural wrist motor control used in everyday life, which is essential for quantifying disorder diagnosis, improving rehabilitation programs, and moving toward more personalized treatments.

Disclosures: A.L. Pando: None. **J.N. Hernandez:** None. **S.K. Charles:** None.

Poster

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VIEP-BUAP SAL103 (EM)

Title: Improved sensorimotor performance via stochastic resonance is related to increased corticomuscular coherence

Authors: C. TRENADO[1], I. MENDEZ-BALBUENA[2,3], E. MANJARREZ[3,4], F. HUETHE[1], J. SCHULTE-MOENTING[1], B. FEIGE[1,2], M.-C. HEPP-REYMOND[4], *R. G. KRISTEVA[1]; [1]Univ. Freiburg, Freiburg, Germany; [2]Facultad de Psicologia, Buap, Mexico; [3]Inst. de Fisiologia, Buap, Mexico; [4]Inst. Neuroinformatics, Univ. Zürich and ETH Zürich, Zürich, Switzerland

Abstract: Internal stochastic resonance is a phenomenon of nonlinear systems that refers to the increase in coherence produced by a particular level of internal or external noise, called optimum noise (ON). The purpose of the present study was to investigate whether the improvement of the performance by ON is related to increased long-range synchronization between motor cortex and muscles reflected in corticomuscular coherence and increased cortical motor synchrony reflected in spectral power (SP). Seven subjects performed a visuomotor task requiring to compensate isometrically with the right index finger a static force generated by a manipulandum on which stochastic noise could be applied. The finger position was displayed on-line on a monitor as a small white dot which the subjects had to maintain in the center of a green bigger circle. EEG from the contralateral motor area, EMG from the active muscles and finger position were recorded. The performance was measured by the absolute deviation from the zero position. The effects of ON were characterized by a better performance, higher cortical motor SP and higher

corticomuscular coherence, as compared to the zero noise condition when no noise was applied to the manipulandum. These data suggest an increase in local cortical motor and long-range synchronization between cortical motor and spinal circuits as the neuronal basis of the improved sensorimotor performance via stochastic resonance.

Disclosures: C. Trenado: None. **R.G. Kristeva:** None. **F. Huethe:** None. **J. Schulte-Moenting:** None. **B. Feige:** None. **I. Mendez-Balbuena:** None. **E. Manjarrez:** None. **M. Hepp-Reymond:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 272.01/AAA1

Topic: D.17. Voluntary Movements

Support: NIH NS074917

Title: Inhibitory processes during response preparation are observed when a muscle is part of the volitional action, but not if the same muscle is recruited for postural stability

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Abstract:
TMS studies have revealed the operation of two inhibitory mechanisms during the selection and preparation of a response. Motor-evoked potentials in the agonist for a forthcoming response (selected) are reduced prior to the onset of an imperative signal, a process referred to as impulse control (IC). MEPs are also attenuated in muscles associated with the non-selected response, a process referred to as competition resolution (CR). Prior studies have provided converging evidence that these inhibitory effects reflect separable processes. Moreover, the effects are limited to task-relevant muscles; they are absent in muscles irrelevant to the competition (Duque et al., 2010). We revisited the specificity question by comparing preparatory changes in excitability in a muscle that was either directly involved in the forthcoming action (e.g., agonist if the finger was selected) or contributed to the action through a required postural adjustment. At the start of the trial, participants used a pinch grip to hold two small objects, one in each hand. This starting state required abduction of the left and right index finger via the FDI. In the volitional conditions, a cue indicated if the object should be squeezed with the left or right hand. In the postural condition, the cue indicated if the object should be lifted with the right or left arm by flexion of the biceps. Here, FDI was modulated in an involuntary fashion as a postural

response to offset slip forces. We used two levels of squeezing in the volitional condition (soft and hard), with the intention to have these forces span the EMG change observed in the postural condition. TMS was delivered over right M1 prior to the onset of the imperative signal, with half of the pulses occurring after a left hand cue (selected) and half occurring after a right hand cue (non-selected). Consistent with previous results, IC and CR in the FDI were observed during the preparatory phase in the volitional condition, regardless of whether the block required soft or hard squeezes. In contrast, neither form of inhibition was observed in the postural condition, despite the fact that there was an increase in FDI due to the increase in grip force. This dissociation indicates that inhibitory mechanisms recruited during response preparation are selectively targeted at muscles that are associated with the volitional component of the action.

Disclosures: L. Labruna: None. **I. Greenhouse:** None. **M. Paluy:** None. **R.B. Ivry:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

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Program#/Poster#: 272.02/AAA2

Topic: D.17. Voluntary Movements

Support: NIH NS074917

Title: Influence of delay period duration on inhibitory processes for response preparation

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Abstract: Transcranial magnetic stimulation (TMS) and peripheral nerve stimulation provide effective tools to probe inhibitory and facilitatory processes during response selection and initiation. When a cue precedes an imperative signal, corticospinal (CS) excitability decreases as the imperative signal approaches, with greater inhibition when the targeted hand is selected than non-selected. These effects are attributed to shaping the selection and timing of the forthcoming response. In the present study, we compared the operation of these processes when the delay was short (300 ms), medium (500ms) and long (900 ms) in a choice RT task (left vs right index finger movement). During the delay period, a single TMS pulse was delivered over right motor cortex to elicit motor evoked potentials in the left first dorsal interosseous muscle. In the medium and long delay conditions, CS inhibition increased over the delay period and was greater when

the targeted hand was selected for the forthcoming response. In the short delay condition, CS inhibition was observed immediately after the cue and was similar regardless of whether the targeted hand was selected or not selected for the forthcoming response. To directly probe excitability changes at the spinal level, median nerve stimulation was used to measure the H-reflex in the flexor carpi radialis muscle. The reflex showed greater attenuation when the targeted hand was selected for the forthcoming response, but only in the long delay condition: the H-reflex response was similar for the selected and non-selected hands in short delay condition. These results demonstrate dynamical constraints in the recruitment of inhibitory processes during response preparation. With short delays, inhibition was recruited in a similar fashion for both selected and non-selected responses, whereas with long delays, inhibition was greater when the targeted hand was selected for the forthcoming response. These results indicate that, when preparation time is limited, all potential responses are inhibited in a similar manner. With longer preparatory intervals, differential mechanisms are recruited to inhibit the selected and non-selected responses.

Disclosures: F. Lebon: None. **C. Papaxanthis:** None. **I. Greenhouse:** None. **L. Labruna:** None. **R.B. Ivry:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

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Topic: D.17. Voluntary Movements

Support: NIH NS074917

Title: Response sequence influences motor excitability during response preparation

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Abstract: Excitatory and inhibitory processes influence the level of motor excitability during the selection and initiation of a motor response. However, little is known about the influence of response history on these processes. We tested whether responses on the preceding trial of a two-choice delayed response task influenced levels of motor excitability during an early and late phase of response preparation. On each trial of the task, participants (n=16) were randomly cued to prepare a lateral abduction of the left or right index finger. After a 900 ms delay, an imperative stimulus instructed participants to execute the selected response. Trials were separated by a 2500-3500 ms inter-trial interval. We applied transcranial magnetic stimulation (TMS) over right

M1 at an early (100ms) and late (800ms) time point during the delay period and measured the amplitude of the resulting motor evoked potential (MEP) from the left first dorsal interosseous muscle, as an index of motor system excitability. To determine the influence of response history on motor excitability, we compared raw MEP amplitudes between the early and late time points when the left hand was selected vs. non-selected to respond during the preceding trial. MEP amplitude was significantly greater at the early than at the late pulse when the left hand was selected to respond during the preceding trial. However, there was no significant difference between the two time points when the left hand was not selected on the preceding trial. Moreover, this pattern of results was insensitive to whether the left hand was selected or not selected on the current trial. This result suggests that modulation of motor excitability over the course of a delay period depends upon the response executed on the preceding trial. This finding has implications for understanding the time course of excitatory and inhibitory processes that operate during the preparation of a response, as well as methodological implications for choosing appropriate inter-trial intervals when designing TMS experiments.

Disclosures: I. Greenhouse: None. **F. Lebon:** None. **L. Labruna:** None. **C. Papaxanthis:** None. **R. Ivry:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: Wellcome Trust

Marie Curie Postdoctoral Fellowship (S.W.)

UCL Grand Challenge (G.V.)

Title: Modulation of the intra-cortical LFP during action execution and observation

Authors: *S. WALDERT[1], R. PHILIPP[1], G. VIGNESWARAN[1], J. M. KILNER[1,2], R. N. LEMON[1], A. KRASKOV[1];
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Abstract: It is well established that the neuronal activity in cortical motor areas can be changed by observation of movements without execution of actual movements by the observer. Human

EEG and MEG studies reported a decrease in the power of beta oscillations in the motor cortex during movement observation similar to the well-known phenomenon of beta power reduction during movement execution. A special class of motor neurons, called mirror neurons, which modulate their activity both during movement execution and observation of the same movement performed by the experimenter, were originally discovered in macaque ventral pre-motor cortex (PMv), area F5. This study focuses on mirror properties of the intra-cortical local field potential (LFP), which reflects the superimposed, mainly synaptic activity of neuronal clusters.
Using single electrodes and electrode arrays, we recorded intra-cortical LFPs in macaque PMv, M1 and areas on the convexity of the pre-central gyrus between PMv and M1. Monkeys were trained to reach, grasp, hold and release different objects and to observe the same actions performed by a human experimenter. Arm and hand EMGs were recorded simultaneously with LFPs.
We found that neuronal population activity in PMv and M1, reflected by the intra-cortical LFP, can be modulated by action observation. Importantly, no overt EMG activity was present during observation indicating that no covert movements were executed.
LFP modulations during observation were found in the low-pass filtered LFP (<7 Hz, the movement related potential (MRP)) when the experimenter grasped an object but could also be detected during object release. Beta activity decreased mainly during observation of the grasping movement and increased during observation of the hold period. Modulations in high-gamma were partly observed during observation of the grasping movement.
The modulations of the MRP during action observation were similar but weaker than those recorded during action execution. Decoding LFPs during action execution showed that M1-LFPs carry more information about grasp type than F5-LFPs. During action observation, decoding accuracies for the LFPs from both areas were lower but similar for both areas.
These findings might indicate that both F5 and M1 are involved in processes mediated by mirror activity. In addition, the presence of modulations in the low-pass filtered LFP during action observation corroborate the importance and the genuine neuronal origin of this signal component, which has been shown to be a suitable input signal for Brain-Machine Interfaces (BMI) because it carries substantial information about movement parameters in LFP, ECoG, EEG and MEG signals.

Disclosures: S. Waldert: None. **R. Philipp:** None. **G. Vigneswaran:** None. **J.M. Kilner:** None. **R.N. Lemon:** None. **A. Kraskov:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 272.05/AAA5

Topic: D.17. Voluntary Movements

Title: Effector coding for motor planning: Dynamic activation and information topologies in posterior parietal cortex

Authors: *F. LEONE[1], T. HEED[2], I. TONI[3], W. MEDENDORP[3];
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Abstract: Neuroimaging in humans and neurophysiology in monkeys have suggested segregated processing in the posterior parietal cortex (PPC) for saccades and reaches. These findings have been interpreted to indicate an effector-specific organization of the PPC. However, when comparing eye, hand, and foot motor planning in humans using fMRI, we recently found that the activation in the PPC did not differ between hand and foot motor planning, whereas limb activation segregated from saccade activation (Heed et al. J Neuroscience 2011), suggesting a functional rather than an effector specific organization. Recent multivariate pattern analysis studies, however, have shown that effector-specific information (difference in the pattern of activation across voxels) can reside within the same brain area without showing effector-specific activation (difference in average activation across voxels). Here, we apply a combination of activation and information measures to explore the organization of the PPC.
Sixteen participants performed delayed (1.6-5.6 sec) goal-directed eye, right hand, or right foot movements in an fMRI scanner. Data were analyzed using a novel method combining effector-specific activation and information measures. To this end, search-spheres (radius: 2 voxels) were used to estimate both effector-related average activity and correlations between effectors, separately for stimulus-, delay-, and movement-related epochs in each trial. The activation index represented the magnitude of the effector-related effects; the correlation index characterized the independent information provided by those effector representations. Based on these indices, we revealed the effector-specific topologies (differentiating each effector from the other two) and functionally-specific topologies (differentiating eye and limb representations) across the cortical surface.
Large portions of PPC had a functionally-specific organization, distinguishing eye and limb, primarily during the delay period. In contrast, the anterior parietal cortex demonstrated an effector-specific organization. During the stimulus and movement periods, anterior parietal cortex as well as pre- and supplementary motor areas, including the frontal eye fields, showed effector-independent activations. We conclude that PPC contains a functional gradient in both activation and information, which is employed specifically during the planning of movements.

Disclosures: F. Leone: None. **T. Heed:** None. **I. Toni:** None. **W. Medendorp:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: NIH/NINDS Grant: NS053962

NSERC Postdoctoral Fellowship

Title: The cortical bases of hand selection for object manipulation in humans: Insights from fMRI repetition suppression

Authors: *K. F. VALYEAR, S. H. FREY;
Dept. of Psychological Sci., Univ. of Missouri, Columbia, MO

Abstract: Choosing which hand to use to perform actions is one of the most commonplace decisions we make as humans, and yet very little is understood about how the brain resolves these choices. Here we used a novel fMRI repetition suppression (RS) paradigm to identify brain areas important for hand selection for object manipulation. *Methods.* Novel tools were presented to participants in the MRI scanner with their handles oriented vertically, facing downward with respect to the workspace. According to arbitrary rules defined by the shape of tools, participants used either their left or right hands to reorient tools so that their handles faced either to the left or right. To rotate tools to the left versus right participants performed distinct types of hand actions (Action Types) involving distinct movements, and this was true for actions made with either hand. Critically, individual trials comprised two actions - a prime and a probe, separated by a 2.5s delay interval. Four conditions were defined by the relationship between prime and probe: either the same actions were repeated (Identical Repeat, IR), hand was repeated but action type was changed (Hand Repeat, HR), action type was repeated but hand was changed (Action Type Repeat, ATR), or neither hand nor action type were repeated (No Repeat, NR). Trials were separated by 15s intervals. *Results*. Response times to initiate actions were faster for IR versus all other conditions, and for HR versus both ATR and NR conditions. While the former result may be attributable to the repetition of hand, action type, tools and/or tool-defined action rules, the latter result is attributable to hand repetition. We take this to reflect more efficient action planning when the same hand can be used for successive actions, independent of the movements involved in those actions. Bilateral parietofrontal and left-lateralized occipitotemporal cortex showed RS for IR versus all other conditions (IR < HR, ATR, NR). A subset of these areas, namely bilateral posterior intraparietal and cingulate as well as left dorsal premotor cortex, showed RS for HR versus ATR and NR conditions (HR < ATR, NR). These results are interpreted as evidence for abstract hand-specific representations for action planning in these areas. We hypothesize that hand selection is resolved by biasing the competitive activity between

these hand-specific representations. According to this view, the current results identify recent hand selection history as an important source of selection bias. When the same hand can be used for successive actions, competition within these areas is more efficiently resolved, and both response times to initiate actions and fMRI activity levels are reduced.

Disclosures: K.F. Valyear: None. **S.H. Frey:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: EU Grant FP7-ICT 217077-EYESHOTS

Fondazione del Monte di Bologna e Ravenna (Italy)

MIUR

Title: Body-centered, mixed, but not hand-centered coding of visual targets in the medial posterior parietal cortex during reaches in 3D space

Authors: *K. HADJIDIMITRAKIS[1,2], F. BERTOZZI[1], R. BREVEGLIERI[1], M. G. ROSA[2], C. GALLETTI[1], P. FATTORI[1];
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Abstract: The posterior parietal cortex (PPC) is crucially implicated in the reference frame transformations that underlie visually guided arm reaching movements. The classical view on these transformations holds that
each PPC area represents spatial information in a specific system of coordinates depending on its relative proximity to sensory input and motor output, so that caudal PPC areas use eye-centered and rostral areas hand-centered coordinates. Accordingly, the information about target location, while being transferred along the caudorostral PPC axis, is gradually transformed from eye- to hand-centered reference frames. However, numerous recent studies challenge this view by demonstrating that multiple reference frames can be present in the same region and that large populations of neurons in several PPC areas use intermediate frames of reference. In all these studies arm movements were performed to targets arranged on a frontal plane, so the presence of multiple reference frames and intermediate representations in 3D space is still an open issue. We

addressed this issue in the caudal PPC area V6A, where it had been shown previously that reach targets located on a single plane were encoded reach in an intermediate eye- and body-centered coordinates by most of the neurons, with smaller populations of cells using "pure" eye- or body-centered frames of reference. In the present study, we manipulated the initial hand position to test between body- and hand-centered representations. Single unit activity was recorded from V6A in two *Macaca fascicularis* monkeys while they performed reaches in darkness towards targets located at different distances and lateralities from the body. We found two major populations of V6A cells: a) neurons that encoded targets in intermediate body and hand-centered coordinates, and b) cells not affected at all by stating hand position, which used "pure" body-centered representations of target location. Interestingly, we only found very few neurons that represented targets in "pure" hand-centered coordinates, despite the fact that the manipulation of hand position in depth was expected to increase the influence of proprioceptive signals from the hand. These findings argue in favour of area specific reach representations in PPC. Our results also provide for the first time evidence that intermediate frames of reference are widely used to represent targets in depth.

Disclosures: K. Hadjidimitrakis: None. **F. Bertozzi:** None. **R. Breveglieri:** None. **M.G. Rosa:** None. **C. Galletti:** None. **P. Fattori:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Title: Reaching related neurons in the Superior Parietal Lobule are strongly modulated by hand-positional signals when movement is directed to memorized targets

Authors: *E. BRUNAMONTI, A. GENOVESIO, M. A. GIUSTI, R. CAMINITI, P. PANI, S. FERRAINA;
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Abstract: In a previous report using a delayed reaching-in-depth (RID) task (Ferraina et al., J Neurosci 2009) we showed that reaching related neurons in parietal area PE are modulated by target depth and that the initial hand position had a strong effect on the neural activity as well. Most important, we provided evidence that PE modulation was mainly explained by the component in depth of the motor error, i.e., the vector of the planned arm movement that depends on the representation of both arm position and target location in space. Here, by

comparing data obtained in both a visual and memorized versions of the task we examined the role of the hand-related signal in relation to whether the target was visible or not during motor preparation.
Monkeys were trained to reach towards either visual (RIDvis) or memorized (RIDmem) targets located at different distances, after a delay of variable duration. Of 179 neurons recorded from area PE in both tasks, 116 (69%) were classified as reaching related of which ninety-four (81%) were modulated by changes in the initial hand position. Additionally, 38 (40%) of the hand-modulated neurons were influenced by the task.
We estimated the time in which the hand position information started to be used differently in the RIDmem and RIDvis respectively and, within each task and behavioral epoch, we quantified the influence of the hand initial position on the neural discharge. For each neuron we first identified the initial hand position associated to the highest activity in both tasks, and then we performed a time dependent analysis calculating the area under the ROC (AUROC) to identify when the activity in the two tasks started to differ. On average the hand modulated neurons started to use differently the hand position signal, 455 (64) ms before the movement onset. In the last 300 ms of the delay epoch we computed the AUROC for the hand positions in each task finding a 10 % of decrease in accuracy in estimating the initial hand position in the RIDvis compared to the RIDmem. Overall these results indicate that the hand position signal is less important for the PE's neural computation of the motor plan toward visible than memorized targets and highlights the flexibility of this parietal area to weight the available contextual information.

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Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: Wellcome Trust

UCL Grand Challenge

Title: The influence of gaze on the activity of macaque M1 and F5 mirror neurons

Authors: *R. PHILIPP, G. VIGNESWARAN, S. WALDERT, R. LEMON, A. KRASKOV; Sobell Dept. of Motor Neurosci. & Movement Disorders, Box 28, UCL Inst. of Neurol., London, United Kingdom

Abstract: Paying visual attention to an object which is going to be manipulated is an essential requirement for most tasks performed in everyday life of primates. In the mirror neuron literature, it is generally assumed that the observer is paying attention to what the actor is doing. Here, we will present data showing that this is indeed the case. We correlate the gaze behaviour i.e. the time spent by a macaque monkey looking at the object, with the neuronal firing rate of M1 and F5 mirror neurons while the monkey executed visually-guided grasps, and during observation of the same grasps mirrored by a human experimenter.
We monitored the eye movement pattern of the monkey by means of a non-invasive infra-red eye tracking system (ISCAN ETL-200, 120Hz). The monkey executed and observed three different types of skilled grasp: precision grip (trapezoid object), hook (small ring) and whole hand grasp (sphere). After an inter-trial waiting period, one of the three objects was presented either in the monkey's peripersonal space (execution trials) or in its extrapersonal space (observation trials). After a short observation period (epoch 1, presentation), a green light around the object (Go cue) (epoch 2, reaction time) instructed either the monkey, or the experimenter sitting opposite the monkey, to reach out and grasp the object, displace it against a spring load (epoch 3, movement), and hold it for 1s (epoch 4, hold), and then release it. The task did not require any particular eye movement or fixation, which allowed us to investigate the natural eye movement pattern during both execution and observation of the task.
Compared to its own actions, the monkey spent less time looking at the object during observation than during execution. However, the object fixation patterns between both conditions were highly correlated (0.92, $p<0.05$) emphasising that the monkey paid attention to the experimenter's actions during observation trials although this was not explicitly required in the task design. We simultaneously recorded mirror neurons in M1 (n=18) and F5 (n=36) in 28 sessions. Comparing neuronal firing rates for trials when the monkey attended the object for longer vs. shorter periods of fixation revealed that during observation trials 19% of the 54 recorded mirror neurons exhibited a higher discharge rate during epoch 3 the longer the monkey spent looking at the object while 9% showed the opposite effect. During execution trials 10% of the units were accounted for each of the effects.
The overall finding is that there are gaze-related changes in the activity of M1 and F5 mirror neurons, but many mirror neurons do not show any gaze-dependent activity changes.

Disclosures: R. Philipp: None. **G. Vigneswaran:** None. **S. Waldert:** None. **R. Lemon:** None. **A. Kraskov:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: MIUR

FP7 - ICT 217077 - EYESHOTS

Fondazione del Monte di Bologna e Ravenna

Australian Research Council

Title: Precuneate cortical connections in the macaque monkey

Authors: L. PASSARELLI[1], *S. BAKOLA[2], M. GAMBERINI[1], K. J. BURMAN[2], K. E. RICHARDSON[2], P. FATTORI[1], M. G. P. ROSA[2], C. GALLETTI[1];
[1]Dept. of Pharm. and Biotech., Univ. of Bologna, Bologna, Italy; [2]Dept. of Physiol., Monash Univ., Clayton, Australia

Abstract: The posterior parietal cortex in the macaque precuneate region (BA 7m, or PGm) has remained relatively unexplored, in part due to difficult access. Recent human imaging studies have ascribed a multitude of visuospatial and cognitive functions to this field, suggesting possible anatomical heterogeneity, and detailed histological analysis in macaque monkey demonstrated distinct rostral and caudal cytoarchitectonic fields within area PGm. The aim of this study was to examine the cortical connections of PGm, including possible variations in projections to different sectors of this region. Seven retrograde fluorescent tracers were placed in PGm in three macaque monkeys anesthetized with alfaxan (10 mg/kg). The distribution of labelled cells was visualized with fluorescence microscopy, and presented in computer-assisted reconstructions of the cortical surface. The principal sensory input to the PGm as a whole arrived from high-order medial extrastriate cortices (V6Av, and a yet to be characterized area located adjacent to peripheral V2), from oculomotor-related areas of the inferior parietal lobule (PG, Opt), and from visual area MST. Strong projections also stemmed from caudal cingulate area 23. Frontal projections arose mainly from the dorsal premotor cortex (F7), and from prefrontal areas 8 and 46. Rostral PGm received additional somatomotor input, from superior parietal areas PEci and V6Ad, whereas caudal PGm received stronger visual inputs. The present results indicate that PGm receives consistent input from high-order sensory, motor, and limbic cortices, in line with the proposed associative role of the area. The regional variations in the architectural structure and connectivity patterns likely reflect functional specializations within PGm.

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Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Program#/Poster#: 272.11/AAA11

Topic: D.17. Voluntary Movements

Title: Probing corticospinal excitability at point of object contact during constrained vs. unconstrained grasps

Authors: *M. DAVARE[1], P. PARIKH[2], P. MCGURRIN[2], M. SANTELLO[2];
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Abstract: For successful object manipulation, the brain needs to precisely scale fingertip forces based on a prediction of digit positioning. When lifting an object with an asymmetrical center of mass, it has been shown that load force development varies as a function of digit positioning on a trial-by-trial basis. The question arises as to how information about digit positioning shapes corticospinal output, which in turn generates an appropriate load force pattern. To address this issue, we asked subjects to lift without tilting an object with an asymmetrical center of mass. Subjects could grasp the object either at self-chosen (unconstrained) or predetermined (constrained) locations. Our rationale was that unconstrained and constrained grasps would require the primary motor cortex (M1) to assign a different weighting to sensory feedback about digit positioning since these grasp conditions rely on a different balance between open- vs. closed-loop motor control mechanisms. To do so, we probed corticospinal excitability (CSE) at different time points, during grasp planning and at object contact, by using single pulse transcranial magnetic stimulation (spTMS) with an intensity of 80 and 120% of resting motor threshold, respectively. Subjects (n=7, right-handed) lifted the object using a precision grip (thumb-index) in an unconstrained or constrained fashion in two pseudorandomly distributed blocks. Digit positioning in the constrained condition was adapted to each subject's average digit placement, measured during practice trials prior to the experiment, so as to ensure any CSE change was not due to simple hand shaping differences. We found that when spTMS was delivered at object contact, CSE was greater for the unconstrained compared to the constrained grasp condition, although digit positioning was identical in both conditions. There was no difference in CSE for these two grasp conditions prior to object contact, i.e. during grasp planning or in the middle of the reaching phase. This shows evidence that M1 integrates context-dependent sensorimotor information about digit positioning differently at object contact, a time point when processing of online feedback is crucial for controlling fingertip forces.

Disclosures: M. Davare: None. **P. Parikh:** None. **P. McGurrin:** None. **M. Santello:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 272.12/AAA12

Topic: D.17. Voluntary Movements

Support: Geconcerteerde Onderzoeksacties (GOA 2010/19)

Fonds voor Wetenschappelijk Onderzoek Vlaanderen (G.0495.05, G.0713.09)

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Interuniversity Attraction Poles 7/

ERC-StG-260607

Title: Visual monitoring of hand actions in AIP neurons

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Abstract: The macaque Anterior intraparietal area (AIP) is involved in the visuomotor control of grasping . Recent data (Pani et al., Program No.852.08 Society for Neuroscience 2011, Abstract viewer, online) showed a convergence between visual and motor responses in single AIP neurons, i.e. the same neurons fired both during grasping execution and grasping observation (grasping and observation neurons). This type of neurons could be involved in the monitoring of the interaction of the hand with the object . To investigate this hypothesis we selected grasping and observation neurons using a delayed visually-guided grasping (VGG) task (to test their motor properties) and an observation task in which the monkey observed a movie showing a grasping action in 1st person perspective (to test for visual responses). We selected 51 AIP grasping and observation neurons out of 183 neurons recorded. These neurons were further tested in one out of two observation tasks: a static hand observation task (SHOT), and a dynamic grasping observation task (DGOT). In the SHOT 4 static images of different phases of the grasping action were presented (object alone, hand reaching, hand on the object, hand holding the object).We found that 14/29 neurons (48%) detected the presence of the hand on the scene (Global aspect) by distinguishing between the object alone and at least one of the other three conditions. Furthermore almost all of them distinguished between at least two different hand configurations on the monitor (13/14, Detailed aspect). We compared their ability to distinguish between global and detailed aspects by comparing the Effect size obtained for each of the 14 selective cells. Overall the neurons were more strongly modulated by Global aspect (Es:1.37 vs 0.9, p=0.01).
In the (DGOT) a movie started showing a grasping hand moving toward the object to grasp. When the hand was covering the object but not yet grasping it (changing frame) the action evolved in 1 out of 4 possible actions, divided in two categories: hand absent (hand retracting or

disappearing) and hand present (hand still or grasping).We recorded 21 cells and contrasted their mean firing rates in the 500ms following the changing frame. Most neurons (18/21) distinguished between conditions with hand present or absent (Global aspect). Of those 10 neurons were also able to distinguish between conditions inside each category (Detailed aspect). Also in this task the cells were more able to distinguish between global than specific aspects (Effect sizes: 1.15 vs 0.72, p=0.002).
Thus, grasping and observation neurons in AIP can monitor the grasping action in terms of both global and specific aspects, but global aspects are more represented.

Disclosures: P. Pani: None. **T. Theys:** None. **P. Janssen:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Support: ERC-StG 260607

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FWO grant G.0713.09

Title: The functional connectivity of macaque area AIP

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Abstract: A previous electrophysiology study identified two separate locations in monkey area AIP which contained neurons selective for disparity-defined depth structure: one located more anteriorly (aAIP), and a posterior spot (pAIP) neighboring area LIP (Van Dromme et al., SFN 2012, New Orleans, 264.03/Z14). The goal of our current research was to examine the functional connectivity of both stereo-sensitive AIP spots using electrical microstimulation during fMRI. In interleaved blocks, two macaque monkeys performed a memory-guided saccade task and a passive fixation task. Stimulation blocks for both tasks were interleaved with no-stimulation blocks. During stimulation blocks, area AIP was stimulated in every trial, at 200 micro-amperes.

Stimulation started together with target or distractor onset, and lasted for 500 ms (pulsewidth: 0.48 ms, frequency: 200 Hz). Both monkeys were injected with a contrast agent and scanned at a 3T Siemens MR scanner with an 8-channel phased-array coil. Before every scan session, a platinum-iridium electrode (impedance: 40-150 kΩ) was inserted in the recording grid, and fixed at a depth which had previously been determined based on single- or multi-unit responses. We analyzed the effects of microstimulation by comparing stimulation blocks with no-stimulation blocks averaged across tasks (memory saccades and passive fixation).
Electrical microstimulation of the most anterior part of area AIP (aAIP) caused increased fMRI activations in the stimulated area itself and in a network of areas implicated in reaching and grasping: the medial bank of the intraparietal sulcus corresponding to area MIP (the parietal reach region); somatosensory area S2; parietal areas PFG and 7a and premotor area F5. Similar results were obtained when stimulating aAIP under ketamine/medetomidine anaesthesia (1mA, 250 ms stimulation).
A largely different pattern of activations was observed when stimulating more posteriorly located pAIP: we obtained increased fMRI-activation in pAIP, in area TEO and in the Caudal Intraparietal area (CIP), while no stimulation-induced activation was obtained in area MIP, and only modest increased activation in premotor area F5. Similar results were obtained in anaesthetized animals. Furthermore, the results were verified in a third monkey in which pAIP was previously identified by the presence of selective single-unit responses to images of objects.
Our results thus suggest that area AIP consists of two subdivisions with different functional connectivity: while aAIP is embedded in a network of areas implicated in reaching and grasping, pAIP is connected with areas typically more implicated in object processing.

Disclosures: E. Premereur: None. **I.C. Van Dromme:** None. **W. Vanduffel:** None. **P. Janssen:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

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DARPA N66001-10-C-2009

Title: The causal role of the posterior parietal cortex in online feedback control of visually-guided reaching movements

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Abstract: The parietal reach region (PRR) in the posterior parietal cortex (PPC) consists of neurons that are activated selectively during planning and execution of reaching movements. Inactivating the macaque PRR impairs reaches to eccentric visual targets, the major symptom of human optic ataxia (OA) caused by lesions in PPC (Hwang et al., 2012). Taken together, these findings suggest that PRR, a functionally defined area within PPC, may be the critical site for OA.

Besides misreaching to static visual targets, OA patients fail to make smooth adjustments when the target unexpectedly jumps to a new location during the course of a reaching movement. The degraded in-flight adjustments are an indication of an impairment of the feedback control system that updates motor commands based on the difference between the current hand location and the new target. It is unknown whether the same neural substrate contributes to both misreaching and diminished online feedback control in OA, or alternatively whether the two classes of deficits are observed together due to lesions spanning PRR and adjacent areas.

To investigate this issue, we inactivated PRR using muscimol and examined its effects on two non-human primates' movements in a target jump task in which the visual target unexpectedly jumped to a new location upon reach onset. We found that PRR inactivation affected reach endpoints in target jump trials similarly to reach trials with static targets, i.e., reaches ended short of the jumped target. Under PRR inactivation,

movement duration and the peak velocity decreased, in proportion to the shorter movement length. However, the frequency of trials lacking in-flight adjustments, reaction time to the target jump, and the variability of trajectories remained unchanged. This is in contrast to degraded online movement control resulting from area 5d inactivation, as indicated for example by delayed trajectory correction.

In summary, PRR inactivation did not abolish online feedback control, but affected it by distorting the difference vector between the current hand location and the new target location. Our results suggest that PRR represents a subset of neural circuits affected in OA, and that the lack of automatic feedback control is attributable to other PPC areas, with area 5d being a possible candidate.

Hwang EJ, Hauschild M, Wilke M, Andersen Richard A (2012) Inactivation of the parietal reach region causes optic ataxia, impairing reaches but not saccades. Neuron 76:1021-1029.

Disclosures: E. Hwang: None. **M. Hauschild:** None. **R.A. Andersen:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

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Program#/Poster#: 272.15/AAA15

Topic: D.17. Voluntary Movements

Title: Corticospinal excitability during planning of a dexterous grasp

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Abstract: Humans are able to coordinate the amount of finger forces applied on an object in relation to where it is grasped for successful manipulation. However, the neural mechanisms underlying the planning of these grasp parameters are unknown. Furthermore, it is not clear *what* is being planned prior to grasp execution. In this study, we probed corticospinal excitability (CSE) using single-pulse transcranial magnetic stimulation (spTMS) over the primary motor cortex (M1) during two tasks. Young right-handed subjects (n = 11) were visually-cued to plan digit placement on the object at specific locations followed by exertion of either negligible (task "position"; P) or 10% of their maximum pinch force (task "position and force"; P&F) on the object. We hypothesized that planning to perform these tasks influences CSE differently prior to the grasp onset. To quantify the temporal evolution of this modulation, we delivered spTMS over left M1 at one of eight latencies from the '*task*' cue in a random order: 500, 750, 1000 ('*go*' cue), 1100, 1200, 1300, 1400, and 1500 ms. Consistent with previous literature (Cattaneo et al, 2005; Prabhu et al, 2007), the CSE assessed during movement preparation but prior to reach onset was suppressed when compared with the resting state CSE. Moreover, modulation of the CSE based on task characteristics was observed when the TMS pulse coincided with the signal to initiate the grasp, i.e., the '*go*' cue at 1000 ms after cue presentation. Specifically, significantly greater suppression of motor-evoked potentials, assessed in the first dorsal interosseus ($p = 0.001$) and abductor pollicis brevis ($p = 0.053$) muscles, was observed when subjects planned for task P&F versus task P. Similar task-dependent effects were not observed in abductor digit minimi and flexor carpi radialis muscles. In a control experiment, we demonstrated that this task-dependent modulation in the CSE was not related to the amount of force planned and applied to the object following contact. Overall, our findings suggest that planning force following contact versus making contact with an object engages different brain networks and/or reflects higher gain of inputs from the same brain network to M1. Future work will assess the contribution of premotor and anterior intraparietal areas on CSE during planning of dexterous manipulation.

Disclosures: P.J. Parikh: None. **P. McGurrin:** None. **M. Davare:** None. **M. Santello:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: NSERC-Natural Sciences and Engineering Research Council of Canada

CIHR-Canadian Institutes of Health Research

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Title: Selective modulation of interactions between areas of the dorsomedial pathway during the transport and grip formation of goal-directed hand actions

Authors: *M. VESIA[1], M. BARNETT-COWAN -[2], B. ELAHI[1], J. L. NEVA[3], M. DAVARE[4], W. R. STAINES[3], J. C. CULHAM[2], R. CHEN[1];
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Abstract: Neural control of goal-directed hand actions is thought to rely on two specialized parieto-frontal pathways: 1) the dorsomedial stream (namely, superior parieto-occipital cortex, SPOC, in humans and the dorsal premotor cortex, PMd) involved in programming arm transport during reaching and 2) the dorsolateral stream (namely, anterior intraparietal sulcus, aIPS, and ventral premotor cortex, PMv) involved in programming hand grip during grasping - both of which send output to primary motor cortex, M1. Recent evidence has argued that macaque dorsomedial stream, specifically V6A (the putative homologue of human SPOC) and PMd, code both hand transport and grip. In humans, functional imaging studies also show evidence that SPOC and PMd differentiate grasping versus touching, but it is not clear whether these brain areas also code the details of the grip. We used dual-site transcranial magnetic stimulation (dsTMS) to test the functional interactions between SPOC-M1 and PMd-M1 in the left hemisphere during different hand actions. Subjects performed an event-related delayed movement task toward a single peripherally located object (consisting of a small cylinder attached atop a larger cylinder). For each trial, after visual presentation of the object, one of three hand movements was instructed: 1) grasp the top cylinder (precision grip); 2) grasp the bottom cylinder (whole hand grasp); or 3) reach-to-touch the side of the object (without preshaping the

hand). We found that motor-evoked potentials (MEPs) were facilitated by SPOC-M1 dsTMS applied at two specific time intervals (150 and 200 ms) after a cue to select a manual response. Specifically, 150 ms after the cue, MEPs were facilitated for all hand actions, whereas 200 ms after the cue, the facilitatory influence occurred only for reach-to-grasp actions, suggesting that over the course of a reach-to-grasp action SPOC-M1 interactions become functionally specific to the process of grip formation. We are currently testing the functional specificity of human PMd-M1 cortical interactions during these hand actions. Consistent with findings reported in the monkey, these results suggest that the human dorsomedial parieto-frontal stream may play a critical role in all phases of reach-to-grasp action. Critically, they challenge the view that the reach and grasp components are processed independently.

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Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: DFG Grant (SCHE 1575/1-1)

Title: Single trial neural correlates of grasping movement preparation in macaque areas AIP and F5

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Abstract: The neural networks of the brain involved in the planning and execution of grasping movements are not fully understood. The network formed by macaque anterior intraparietal area (AIP) and hand area (F5) of the ventral premotor cortex is implicated in the generation of grasping movements. However, the differential role of each area in this fronto-parietal network is unclear. Previously, data collected from these areas were limited to single neuron electrophysiological recordings. Single neuron recordings are not sufficient to elucidate the interaction of neurons at a population level during the formation of a motor plan. To capture network dynamics, we recorded single and multi-unit activity in parallel from chronically implanted electrode arrays in AIP and F5 while two monkeys performed a delayed grasping task (using one of two grip types) that also involved a grip selection component. Implementing the

'initial condition hypothesis' of movement preparation developed by Afshar et al. (2011), we predicted behavior of the animal on a single trial basis. This hypothesis posits that neural population activity prior to movement on a single trial is predictive of the subsequent reaction time. Supporting the results of Afshar et al., who recorded on the border of dorsal premotor cortex and primary motor cortex in a reaching task, we found this method was able to explain significantly more variance in reaction time compared to previously published methods. No difference was found between conditions where the grip type was instructed and when the monkey chose freely. Furthermore, we were able to compare the information content of areas AIP and F5. We found that F5 was able to predict reaction time significantly better than AIP. This result lends support to the hypothesis that AIP represents an earlier step in the visuo-motor transformation and does not well encode the temporal characteristics of upcoming movements.

Disclosures: J.A. Michaels: None. **B. Wellner:** None. **H. Scherberger:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: CIHR Grant

Title: Decoding reveals planning-related signals underlying object grasping and manipulation

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Abstract: Skilled manipulation requires the ability to predict the weights of viewed objects based on learned associations linking the extrinsic properties of an object, such as shape, size and texture, to the intrinsic property of weight. Although it is well established that extrinsic object properties are represented in regions within occipitotemporal cortex (OTC), the neural mechanisms involved in extracting weight information from these properties are unknown. Here, using functional MRI and pattern classification methods, we tested and confirmed the novel hypothesis that object weight is represented in visually defined object-selective regions within OTC. We used an event-related task in which participants first prepared and then executed lifting actions with objects of varying weight. In Experiment 1, participants lifted visually identical objects presented in blocks of trials such that weight could be reliably predicted. In Experiment 2, the same participants lifted objects of varying weight that differed in surface material. Across

both experiments, the activity patterns that formed in object-selective OTC areas prior to movement onset predicted the weight of the object to be lifted and, in Experiment 2, this effect was independent of the mapping between surface material and weight. In addition, we found that activity patterns in material-sensitive OTC regions coded object weight but only when weight could be reliably predicted from material. Our results indicate that the integration of visual and motor-relevant object information occurs at the level of single OTC areas and provide evidence that the ventral visual stream is actively engaged in processing object information for the purposes of action.

Disclosures: J.P. Gallivan: None. **J.S. Cant:** None. **M. Goodale:** None. **J.R. Flanagan:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: CIHR and CRC grants MOP 68812

Title: Neural substrates for allocentric versus egocentric representation in memory-guided reach

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Abstract: The location of a remembered reach target can be encoded in egocentric or allocentric reference frames, but the neural mechanisms for allocentric reach representations are essentially unknown. We utilized an event-related fMRI design to explore the brain regions that support these two types of representation for memory-guided reach. Twelve participants reached with their right hand toward a remembered target location in complete darkness. A reach target and an additional landmark were always presented at the beginning of each trial, at several positions to the left or right of a fixation target. A recorded voice instructed participants either to 1) remember target location relative to the landmark (Allo condition), or 2) ignore the landmark and only remember target location in space (Ego condition). During the following 12s delay period participants had to remember the target location in the appropriate reference frame. In a non-

spatial control condition (Color), participants were instructed to remember (and later report) the color of the target. At the end of the delay the landmark re-appeared at its original or a novel location (left or right). After the landmark disappeared, an auditory go signal instructed participants to reach or report target color according to the initial instruction, and in the Ego condition, participants were signalled to reach toward or opposite to the target. Thus, egocentric motor direction could not be planned during the delay in any task before the go signal. During the delay period, Ego and Allo conditions elicited significantly higher activation compared to the Color control in: bilateral dorsal premotor cortex (PMd), superior parieto-cccipital cortex (SPOC), midposterior intraparietal sulcus, and extrastriate cortex, inferior frontal gyrus, medial frontal gyrus, pre-supplementary motor area in the left hemisphere. The Ego condition produced more activation in bilateral SPOC and right PMd, whereas the Allo condition produced greater activation in early visual cortex. Significant egocentric spatial selectivity (target location relative to gaze) was observed in occipital cortex during the delay, only emerging in the frontoparietal cortices when participants were informed of the reach direction. Significant allocentric spatial selectivity (target location relative to landmark location) was observed in early visual cortex and bilateral inferior temporal gyrus. These results demonstrate 1) a complete parieto-frontal network for reach, 2) a special role for early visual cortex in spatial memory dissociated from reach direction, and 3) a special role for early visual cortex and the ventral stream for allocentric memory.

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Poster

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Topic: D.17. Voluntary Movements

Support: BMBF (BCCN II, Göttingen)

Title: Decoding of movement states and continuous kinematics of hand and arm motions from primate motor, premotor, and parietal cortex

Authors: *V. K. MENZ, S. SCHAFFELHOFER, H. SCHERBERGER;
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Abstract: The primate motor, premotor, and parietal cortex play a crucial role in the planning and execution of hand movements. It has been shown that area F5 in the ventral premotor cortex

and the anterior intraparietal area (AIP) carry information about grip type, wrist orientation, and hand shape during movement planning and execution. Here, we are investigating whether AIP and F5, in addition to this categorical information, also encode continuous movements by using spike signals from these areas to detect and predict complete hand and arm movements with 27 degrees of freedom (DOF) and compare these to results obtained with signals from primary motor cortex (M1).

Rhesus monkeys (Macaca mulatta) were trained to grasp and lift ~50 objects of different shape, size, and orientation that were presented in front of them in a delayed grasping task. Hand and arm kinematics were monitored with a sensor glove that can track 27 DOF of all fingers, wrist, and arm joint angles continuously (Schaffelhofer et Scherberger, 2012). At the same time, spiking activity was recorded in parallel with 64 chronically implanted microelectrodes (FMAs, Microprobe Inc.) in each of the areas F5, AIP, and the hand area of M1, and then spike-sorted offline (WaveClus, Plexon Offline Sorter). From these population spike data, we detected time periods during which the monkey rested or moved its hand or arm using a weighted support vector machine. Furthermore, we predicted continuous hand and arm kinematics with a Kalman filter.

Classification of movement vs. resting could be done almost equally accurately from signals in M1 or F5 (96% correct classification with M1, 95% with F5), whereas movement detection was significantly worse from AIP (85% correct). When decoding continuous hand and arm angles, differences between M1 and F5 became more apparent: the mean correlation coefficient (CC) (± standard deviation) between real and decoded trajectories across all 27 DOF (across 2 sessions of a first animal) was 0.74±0.14 when using signals from M1 for decoding, whereas the CC was 0.64±0.16 for signals from F5 and 0.55±0.16 for AIP. In contrast, we determined a chance-level CC of -0.005±0.023 when spiking data was temporally shuffled so that the causal link between movement and neural activity was destroyed.

These results indicate that both F5 and AIP carry information about continuous hand movements and movement timing, whereas AIP is less suited for continuous movement predictions. These findings suggest a fundamental difference in the coding of continuous movement information in AIP and the (pre-) motor areas F5 and M1, which could be useful for future hand prosthetic designs.

Disclosures: V.K. Menz: None. **S. Schaffelhofer:** None. **H. Scherberger:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Topic: D.17. Voluntary Movements

Support: MIUR of Italy, PRIN 2010 prot. n. 2010XPMFW4_004 to Alexandra Battaglia-Mayer

Title: Local field potentials are influenced by cooperative joint-action in frontal and parietal cortex of macaque monkeys

Authors: *O. PAPAZACHARIADIS, S. FERRARI-TONIOLO, F. VISCO-COMANDINI, R. CAMINITI, A. BATTAGLIA-MAYER;
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Abstract: The importance of social interaction in human and cultural evolution is only surpassed by the extreme complexity of social brain functions, making neural processes underlying social behavior difficult to study. Actions are identified as own or alien and in some cases alien actions trigger the same neural activity as own actions. However, evidence of neuronal activity associated to joint action in a social context is scant.
We studied extracellular local field potentials (LFPs:1-100 Hz) from premotor cortex (PM) and inferior parietal lobule (IPL) in two rhesus monkeys, during a joint-action task. Our goal was to determine not only the relationship between behavior and LFPs, but more importantly the context influence of an obligatory joint-action with a partner on neural activity.
Monkeys were trained in a center-out task in two conditions. In the first condition (SELF) each monkey, individually, had to move its cursor from the center toward a peripheral target, by applying a force on an isometric joystick, while the partner monkey observed the action. In the second condition (cooperative joint action: COOP), both monkeys had to move their cursors simultaneously toward the same peripheral target, constrained by a maximum inter-cursor distance limit visualized as an outlined circle encompassing the two cursors. Thus, in this condition they had to cooperate to reach a common goal.
We recorded neural activity from 236 PM and 166 IPL sites together with behavioral key events, simultaneously from homologous areas of both monkeys, by using two multiple-electrode arrays. Offline, we defined two epochs of interest, reaction time (RT: 0.2 s from target onset) and movement time (MT: 0.2 s from movement onset). For each trial we calculated the peak-to-peak LFP amplitude in each epoch. Finally we compared these values between the self-acting (SELF) and joint acting (COOP) and between peripheral target directions.
Behavioral analysis of reaction time and movement time showed that monkeys adapted their behavior during the joint-action condition in order to accommodate the partner's behavior. A two way ANOVA showed a significant difference of LFP activity during RT or MT between SELF and COOP conditions in 25.8 % PM and 21.1% IPL sites and between peripheral target directions in 41.9% PM and 36.7 % IPL sites with an interaction factor that resulted significant in 11% PM and 13% IPL sites.
Our data show that there exists in the parieto-frontal system an action cooperation network which is set in motion during cooperative joint-action. We also show that LFP, reflecting cell assembly coordination, can disclose executive and higher-order neural processes.

Disclosures: O. Papazachariadis: None. **S. Ferrari-Toniolo:** None. **F. Visco-Comandini:** None. **R. Caminiti:** None. **A. Battaglia-Mayer:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 272.22/AAA22

Topic: D.17. Voluntary Movements

Support: MIUR of Italy, PRIN 2010-11 prot. n. 2010XPMFW4_004

Title: Neural activity associated to joint-action during social cooperation in frontal and parietal cortex of macaque monkeys

Authors: *F. VISCO-COMANDINI, S. FERRARI-TONIOLO, O. PAPAZACHARIADIS, R. CAMINITI, A. BATTAGLIA-MAYER;
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Abstract: The neural mechanisms related to the ability of humans and non-human primates to interact through joint-action are still poorly investigated. In the domain of motor functions, the study of goal-directed movement showed that no obligatory relationship exists between neural activity and movement, but rather movement-related activity is context-dependent and linked to different cognitive states. So far, neural activity in different cortical areas has been studied in a single brain in action, thus missing all information typical of interacting brains through a joint action task.
Two monkeys sat together in front of a display and they were trained in a center-out task in two intermingled conditions. In the first (SELF), each monkey had to move individually a visual cursor from a central position toward 8 different peripheral targets, by applying a force on an isometric joystick while its partner observed the action. In the second condition (cooperative joint action: COOP), both monkeys had to move their cursors together toward the same peripheral target, under the constraint of a maximum inter-cursor distance limit, which was visualized as an outlined circle incorporating the two cursors. Thus, in this context monkeys had to cooperate to reach a common goal.
Extracellular single-unit activity (SUA) was recorded from premotor cortex (PM) and inferior parietal lobule (IPL), simultaneously from homologous areas of both monkeys by using two multiple-electrode arrays.
Preliminary results showed that kinematic parameters, such as amplitude and direction of the force applied on the joystick, were overall similar in the COOP and SELF conditions. However,

analysis of temporal aspects related to COOP trials showed a tendency of each monkey to adapt its own behavior in order to accommodate partner's behaviour. In 540 PM and 258 IPL neurons studied during Reaction- and Movement Time, a 2-way ANOVA showed a significant difference of SUA between SELF and COOP conditions in 28.5 % PM and 22.1% IPL cells, and between final target directions in 42.6% PM and 39.2 % IPL cells with an interaction factor that resulted significant in 13.7% PM and 14.3% IPL cells. Therefore, in these areas similar actions performed in different social contexts (such as in absence or presence of social interactions), modulates SUA in a different way.
These findings represent a first step toward the description of the neural operations underlying motor functions in a cooperative context and suggest that within this action cooperation network different areas encode joint-action during different behavioural epochs.

Disclosures: F. Visco-Comandini: None. **S. Ferrari-Toniolo:** None. **O. Papazachariadis:** None. **R. Caminiti:** None. **A. Battaglia-Mayer:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 272.23/AAA23

Topic: D.17. Voluntary Movements

Support: NIH Grant 1R01NS076589-01

Title: Reciprocal interactions between distal and proximal upper-limb segments in humans

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Abstract: Previous evidence suggested that the primary motor cortex controls different upper-limb segments as a whole rather than individually. However, electrophysiological interactions between distal and proximal arm segments in the same limb remain poorly understood. In the present study, we examined the effect of voluntary activation of a proximal arm muscle onto the excitability of corticospinal projections targeting distal intrinsic hand muscles. The effect of distal muscle activation onto corticospinal projections of proximal muscles was also tested. Non-invasive transcranial magnetic stimulation was used to elicit motor evoked potentials (MEPs) in the first dorsal interosseous (FDI), abductor pollicis brevis (APB), abductor digiti minimi (ADM), flexor carpis radialis (FCR), biceps brachii (BB), and triceps brachii (TB) muscles while subjects were instructed to complete an isolated ~3-5% maximal isometric voluntary contraction

(MVC) with either the elbow into flexion or the index finger into abduction. To examine the specificity of these effects, MEPs in arm muscles were tested during ~3-5% of MVC with foot dorsiflexors muscles. We found that voluntary contraction with the BB increased MEP size in several of the hand muscles tested including the FDI (by 43.1±25%), APB (by 45.2±27%) and ADM (by 62.5±62%). Similarly, voluntary contraction with the FDI increased MEP size in proximal muscles including the BB (104.9±34%) and TB (44.8±27%). No effects were found of dorsiflexion on MEPS in the muscles tested. Our findings demonstrate bidirectional facilitatory interactions between corticospinal projections targeting distal and proximal arm muscles in intact humans - whether these interactions occur at the level of the cortex and/or spinal cord remains to be determined.

Disclosures: D.S. Soteropoulos: None. **M.A. Perez:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: D.17. Voluntary Movements

Support: NIH Grant 1R01NS076589-01

Title: Ipsilateral corticospinal responses are modulated during bilateral voluntary contractions in humans

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Abstract: Bilateral voluntary contractions involve functional changes in both primary motor cortices. However, the contribution of ipsilateral corticospinal neurons to the control of bilateral upper limb movements remains poorly understood. Here we investigated whether ipsilateral motor evoked potentials (iMEPs) elicited by transcranial magnetic stimulation (TMS) were modulated by bilateral muscle contractions, and whether this modulation was influenced by the direction of the bilateral contraction. Single supra-maximal TMS pulses were given to the left motor cortex during 30% and 100% of maximal isometric voluntary contraction (MVC) with the left biceps brahii muscle into flexion while the contralateral side remained at rest or performed 30% or 100% of MVC into elbow flexion or extension. We found that iMEPs were suppressed during bilateral activation of homologous (biceps-biceps) muscles compared to a unilateral contraction. In contrast, iMEPs were facilitated during bilateral activation of antagonistic

(biceps-triceps) muscles compared to a unilateral contraction. In an additional control experiment, we found that when the head was rotated by 90° leftward (i.e. towards the side of the muscle tested) iMEPs were facilitated. Whereas, when the head was rotated rightward iMEPs were suppressed. The contralateral MEP showed the opposite modulation, suggesting that iMEPs and contralateral MEPs were mediated by different pathways. Our findings support the hypothesis that neural activity in ipsilateral corticospinal pathways is driven, at least in part, by limb kinematics.

Disclosures: T. Tazoe: None. **M.A. Perez:** None.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

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Topic: D.17. Voluntary Movements

Support: NSERC Grant 227920-2010

NSERC Postdoctoral Fellowship

Title: Feedback responses enforce trajectory control when required by the goal of the ongoing task

Authors: *T. CLUFF, S. H. SCOTT;
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Abstract: Trajectory control models were introduced in motor neuroscience almost 30 years ago to explain our tendency to perform straight reaching movements. These models have led to the longstanding view that the motor system controls the desired position or trajectory of the limb during ongoing movements. However, recent studies have highlighted that the motor system compensates for perturbations in a way that maintains task performance rather than produce stereotyped movement patterns. Thus, it is still unclear whether there are circumstances where feedback responses enforce a desired limb trajectory. Here we investigate how the behavioural goal affects feedback responses during upper limb reaching movements. We predicted that feedback responses would only produce stereotyped movement patterns when they are required by the task. Participants (n = 6) performed 10 cm reaching movements with their arm supported in a robotic exoskeleton device (KINARM, BKIN Technologies). The subjects were instructed to reach slowly to the goal target (radius = 1 cm) within 800-1200 ms of movement onset. We probed feedback responses on random trials by applying step torque perturbations that displaced

the elbow during the ongoing reach (±0.5, ±1Nm, ±2 Nm with 10 ms ramp-up profile). Subjects completed two blocks of trials (in random order) where they were instructed either to resist the perturbation and complete the reach within the allotted time (mean reach time = 992±161 ms), or track a target that moved with a bell-shaped velocity profile along a straight path from the start position to goal target in 1000 ms. The radius of the moving target was adjusted for each individual subject based on the variability of his or her nominal (i.e., unperturbed) reaching movements. For each perturbation amplitude and direction (±0.5, ±1, ±2 Nm), we found that subjects reduced their maximum elbow and hand displacements to intercept the moving target before completing the reach, but corrected directly to the goal target when the task did not require precise control of the movement trajectory (all p's < 0.05). In a subsequent experiment, we found that verbal instructions were not sufficient to evoke feedback corrections along a desired trajectory. Our results highlight goal-directed feedback corrections that selectively compensate for errors that influence the outcome of the task. These goal-directed corrections can be modified to produce straight trajectories when required by the ongoing motor task.

Disclosures: T. Cluff: None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies.

Poster

272. **Voluntary Motor Control: Cortical Planning I**

Location: Halls B-H

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Program#/Poster#: 272.26/AAA26

Topic: D.17. Voluntary Movements

Support: NSERC grant 222920-2010

CIHR MOP 84403

Title: Neural correlates of online reaching control in primary motor cortex

Authors: *F. CREVECOEUR[1], T. HERTER[3], S. H. SCOTT[1,2];
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Abstract: It is generally accepted that sensory feedback is essential to control voluntary movements. However, the neural mechanisms underlying feedback control remain highly debated amongst researchers who study voluntary movements such as reaching. One view argues that feedback control is performed by low-level structures (including the spinal cord), which

maintain the limb close to a desired state or trajectory. An alternative hypothesis suggests that the cortical network engaged during motor planning is equally engaged during motor execution, allowing online goal-directed motor adjustments. Here we present data supporting the latter hypothesis. We analyzed firing patterns of neurons in Primary Motor Cortex (M1) of non-human primates performing reaching movements to visual targets (3 monkeys, 117, 306 and 141 cells collected). Monkeys performed centre-out reaching towards one of eight targets uniformly distributed around the starting position. Movement amplitude was 6 cm. We selected cells displaying significant directional tuning during the agonist activity (-50 ms to 100 ms relative to reach onset). This criterion allowed us to include 54 (46%), 137 (44%) and 37 (26%) cells in the analyses, respectively. We concentrated on the reaching movements orthogonal to the cells' preferred directions and compared their activities across trials in which movements initially deviated towards or away from the preferred directions. We reasoned that, if the cell is linked to the generation of movement towards its preferred direction, then it should be engaged in controlling trial-to-trial variability along this axis. We found that the cells' firing rates during the movement (from 0 to 0.5 s relative to reach onset) were greater when the hand was initially moving away from the preferred direction (paired t-test, $t = 2.9$, $P < 0.005$). In addition, the trials displaying greater corrective displacements towards the preferred direction were associated with an increased activity during the reaching movement ($t = 2.7$, $P < 0.01$). Hence, the modulation of single cells' activities during the movement was compatible with the compensation for trial-to-trial variability, and the amplitude of the modulation correlated with the distance travelled along the preferred direction. Altogether, these results support a direct contribution of M1 to online control of reaching movements.

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Poster

272. **Voluntary Motor Control: Cortical Planning I**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 272.27/BBB1

Topic: D.17. Voluntary Movements

Support: NSERC 227920-2010

Title: Rapid Feedback Responses: Rapid switching between multiple goals during movement execution

Authors: *J. Y. NASHED, F. CREVECOEUR, S. H. SCOTT;
Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: Recent work has highlighted that corrective responses to mechanical perturbations consider the shape of behavioural goals (Nashed et al, 2012 JNP). This work highlights the ability to make goal-directed corrections across a spatially distributed goal. However, what if this end goal was a series of discrete goals as opposed to a continuous one? Using a novel obstacle avoidance paradigm we examined if rapid feedback responses differed when reaching to a continuous bar target or multiple discrete dots spanning the same spatial position as the continuous goal.
In the single-long target experiment (SLT), subjects performed reaches (KINARM, BKIN Technologies) from a start target (ST; radius 1cm) close to the body to a bar located directly in front of ST. Participants were instructed to avoid two virtual circular obstacles (radius 1cm) placed strategically to the left and right of the straight path between the start and end goals. Importantly, the obstacles were positioned such that they did not impede the unperturbed reaching trajectories to the center of the end target(s). On random trials subjects were perturbed with 1 of 4 possible joint torques (-0.5,-1,-2 or 0.5 Nm) which displaced the hand leftward or rightward towards the obstacles. The key to the experiment was the medium perturbation (-1Nm), which directed their hand towards the obstacle and forced them to make online decisions. In the multiple target experiment (MLT), subjects performed the same experiment as in SLT, except, the long bar was replaced with three circular targets located at the centre and two ends of the bar's location in the SLT experiment. Obstacles and loads were identical across the two tasks.
In the SLT group, for the medium perturbation (-1Nm), subjects had a diverse mixture of strategies with some movements passing between the obstacles and finishing at the middle of the bar, whereas other movements passed around the obstacles and landed near the end of the bar. We found that long-latency stretch responses (R2 and R3 time periods) were altered across these two strategies, with larger responses generated to direct the hand back between the two obstacles. For the MLT group we found similar patterns of corrective movements for the each perturbation magnitude. However, changes in the long-latency response were found to be only significant during the R3 but not the R2 period. As corrective responses were generated to roughly the same spatial location for the long-bar and the multiple targets, this data suggests that the ability to make corrective responses to a different spatial goal requires additional processing time by the motor system.

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Poster

273. **Cortical Motor Planning: Neuroimaging**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: D.17. Voluntary Movements

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Title: Decoding the representations of grasp types and object properties in the human brain

Authors: *S. FABBRI[1], R. CUSACK[1,2], J. C. CULHAM[1,2];
[1]Brain and Mind Inst., [2]Dept. of Psychology, Univ. of Western Ontario, London, ON, Canada

Abstract: Macaque neurophysiology has suggested a special role of ventral premotor cortex (PMv) and the anterior intraparietal sulcus (AIP) in processing both grasp type and object type. Studies in humans have demonstrated that the putative human homologues of these macaque areas -- PMv and aIPS-- are also involved in the grip component of reach-to-grasp actions and that temporary lesions in these areas cause impairment in preshaping the hand appropriately for the shape and size of the object. It remains unknown to what extent visual and sensory information about shape and size of the objects and the specific grip type are represented and interact in these regions. We recorded human brain activation using functional magnetic resonance imaging (fMRI) and applied multivariate pattern analysis (MVPA). Specifically we utilized a representational similarity approach (Kriegeskorte, Mur, & Bandettini, 2008), to correlate the spatial representations of different grasps and objects in the human brain.
We measured brain activity while participants were passively observing or grasping with 3 grip types (precision grip with 2 digits, precision grip with 5 digits, or a whole-hand grasp) 6 objects (bar, cube, plane, cylinder, sphere, disc) of different sizes (small, medium, and large), for a total of 72 conditions. We collected a rich dataset with 12 repetitions for each of the 72 conditions. We measured how dimensions like task and object size were represented in the human brain by computing correlations between spatial patterns of activation across voxels during odd and even runs in the 72 conditions.
We found that both PMv and aIPS encoded information about the specific task, distinguishing the three grasps from passive viewing. The way in which the three grasps were represented differed between regions: while aIPS showed similar response patterns across all three grasp types, PMv showed greater similarity between the 5-digit and whole-hand grasps than between

either of those grasps and the 2-digit precision grip. Moreover, the representation of the size of the objects depended upon the task, suggesting an interaction between grasp type and object size. Overall, our results indicate that human aIPS and PMv distinguish between grasps to different extents and that this information interacts with information about the size of the object to grasp.

Disclosures: S. Fabbri: None. **R. Cusack:** None. **J.C. Culham:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

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Topic: D.17. Voluntary Movements

Support: Federal Ministry for Education and Science BMBF (FKZ 01GQ1002)

German Research Foundation (DFG CIN)

Title: How far is it? BOLD fMRI activity encodes reach amplitude during planning and execution

Authors: *A. PILACINSKI, A. LINDNER;
Hertie Inst., Tuebingen, Germany

Abstract: Unlike ballistic saccadic eye movements, always executed along stereotyped movement paths, reaching a target with the hand can be realized in various ways. This is needed, for instance, because physical obstacles might be blocking the most direct path. Therefore, as opposed to saccades, the planning of reaching movements needs to also consider movement trajectory information - not just define the movement endpoint. This means that neural activity encoding reaching movements should likewise reflect the basic properties of their trajectories such as movement amplitude. Neural correlates of this component, however, have been difficult to capture especially when using noninvasive brain imaging techniques such as fMRI. We tackled this problem. In a series of experiments we studied reach amplitude effects on BOLD fMRI-signals during movement preparation and execution. All experiments were based upon delayed response tasks, implemented in a MRI-compatible virtual reality reach setup and all statistical analyses were performed on a single-subject basis. In the first experiment we successfully demonstrated a significant modulation of BOLD signals by reach amplitude during both movement planning and execution in posterior parietal and in premotor cortex. In contrast, the hand area of primary motor cortex showed amplitude representation solely during reach execution. Specifically, the larger the amplitude the stronger was the BOLD signal in these areas.

In the second experiment we confirmed these results and moreover we ascertained that the reported differences in BOLD signals do not depend on the eccentricity of target position but instead reliably reflect movement amplitude.

Disclosures: A. Pilacinski: None. **A. Lindner:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

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Program#/Poster#: 273.03/BBB4

Topic: D.17. Voluntary Movements

Title: A role for ventral stream brain areas in understanding errors in tool manipulation

Authors: *J. C. MIZELLE[1,2], R. KELLY[1], L. A. WHEATON[1];
[1]Sch. Applied Physiol, Georgia Tech., ATLANTA, GA; [2]Rehabil. Res. and Develop., Atlanta VA Med. Ctr., Atlanta, GA

Abstract: The successful completion of complex action goals often relies on the use of tools. For this, we must know which tools to use for a specific action goal. Further, we must also know how to manipulate those tools in meaningful way to achieve the goal of the action. Our previous work has highlighted a role for ventral stream brain areas in support of understanding errors in the context of tool use (e.g., identifying that a hammer is not the appropriate tool for stirring coffee). Although clinical observations suggest that dorsal stream areas may be important for understanding errors in the physical usage of tools (e.g., hammers must be grasped and manipulated a certain way to best drive a nail), this has not been fully evaluated in the healthy brain. As such, the purpose of this study was to identify the regions of the brain critical to supporting the process of understanding errors in tool manipulation. Using event-related fMRI, neural activations were recorded while participants silently evaluated whether images showed correct (e.g., hammer held by its handle with the head oriented towards a nail) or incorrect (e.g., hammer held by its handle but with the handle oriented towards a nail) tool manipulation. The context of the action scene was always correct (e.g., hammer used on a nail). We expected similar regional activations (i.e., parietofrontal areas) for both conditions, but with greater activation for identifying incorrect over correct tool manipulation. As expected, both conditions elicited robust activation of parietal and frontal areas. However, greater activation was seen for correct over incorrect tool manipulation along the canonical parietofrontal action network, while activations for identifying incorrect over correct tool manipulation were primarily seen at superior temporal areas and insula. These findings support a fundamental role for ventral brain

areas in functional action understanding. Accordingly, we expand our hypotheses about ventral brain networks identifying contextual error to include mechanisms for understanding functional tool actions, which collectively we regard as functional affordances.

Disclosures: J.C. Mizelle: None. **R. Kelly:** None. **L.A. Wheaton:** None.

Poster

273. Cortical Motor Planning: Neuroimaging

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 273.04/BBB5

Topic: D.17. Voluntary Movements

Title: Effects of verb presentation on motor preparation and motor action

Authors: *M. YUKI[1], S. MORIOKA[2];
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Abstract: Introduction
Humans perform motor actions by their own intentions, and also perform preparation and planning prior to motor action. The efficiency of neural circuits is enhanced by motor preparation, enabling more efficient motor control. “Intention” is important in motor preparation, and language is involved in the formation of “intention”. In fact, verbs which are associated with parts of the body have effects on motor action (Ricardo, 2009). Therefore this study considers whether verb presentation affects the motor preparations.
Methods
The subjects were three healthy adults. The protocol of presentation was as follows: A point of fixation, “×”, was displayed for 5sec and, as the conditioned stimulus (S1), either a hand-related verb or an abstract verb was displayed for 2sec. Following S1, either “Go” or “No” was displayed as the discriminative stimulus (S2) for 1sec. With each trial comprising the period of eight seconds from the display of a point of fixation to the presentation of S2, 100 trials were conducted. With 100 trials as a set, three sets were conducted. Fifty times each of hand-related and abstract verbs were presented as S1. As S2, “Go” was presented 60 times and “No” was presented 40 times. Instruction was given to press a button only when “Go” is presented. Reaction times following the presentation of S2 were measured, and the times for the hand-related verbs and for the abstract verbs were tested by Student’s t-test. EEG measurements were made with a digital electroencephalograph with advanced features, the ActiveTwo system(BioSemi). The record of the EEG assumed 32ch, sampling frequency 1,024Hz, band pass

filter 0.05-50Hz. Event-related potential (ERP) data were taken beginning at 1sec in advance of, and ending 4sec after, the presentation of S1, and signal averaging was carried out for each condition of the hand-related verbs and the abstract verbs. The EEGs were analyzed using EMSE Suite (Source Signal Imaging Inc.), and CNVs as well as changes in the amplitude of N400 were examined.
Results/Discussion
Although there was a tendency for the response time to be short when hand-related verbs were presented, a significant difference was not found ($p<0.05$). The average amplitude of the CNV was large when hand-related verbs were presented, and the average amplitude of N400 was large when abstract verbs were presented. From these results, it is considered that the efficiency of neural circuits is enhanced by the presentation of hand-related verbs, which tends to shorten the response time. Further, it is considered that the mental load of apprehending the meaning is increased by the presentation of abstract verbs, which tends to lengthen the response time.

Disclosures: M. Yuki: None. **S. Morioka:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

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Topic: D.17. Voluntary Movements

Support: Falk Medical Research Trust

Title: Effect of sensory attenuation on cortical movement related oscillations

Authors: *J. LEE, B. D. SCHMIT;
Marquette Univ., Milwaukee, WI

Abstract: Changes in cortical oscillations associated with motor commands after spinal cord injury (SCI) may result from the loss of proprioceptive sensory feedback. After SCI, beta frequency (15-35 Hz) oscillations have been observed to be significantly attenuated compared to control subjects over sensorimotor areas of the cortex. These neural oscillations have been theorized to facilitate communication between different segments of the brain, and their amplitude has been shown to modulate during motor tasks. We hypothesized that changes in amplitude of beta frequency modulation after SCI result from the loss of proprioceptive sensory feedback and that a similar alteration in cortical patterns can be induced in controls with the use of muscle vibration. During this experiment, we measured the effects of attenuated proprioceptive feedback on electroencephalography (EEG) signals in ten young, healthy control

subjects during a simple ankle motor task using prolonged tendon and muscle vibration, previously demonstrated to depress Ia afferents. Participants were instructed to briskly dorsiflex their ankle after a visual cue from a comfortable seated position. Four conditions were randomly interleaved across 5 blocks and consisted of the following: no vibration before the visually cued ankle dorsiflexion, vibration (10 s ± 1 s of 70 Hz) of the tibialis anterior (TA) before the visual cue, electrical stimulation (Estim) of the TA, and vibration of the TA followed by Estim. Signals from a 64 channel EEG actiCAP were sampled at 2000 Hz and band-pass filtered between 1 and 500 Hz. Offline data analysis was conducted using the Fieldtrip and EEGLAB lboxes, and custom Matlab scripts. Electromyography (EMG) recordings (band-passed 10-350 Hz) were taken from the TA and the medial gastrocnemius (MG) muscles. A beta band time frequency (TF) decomposition of the Cz electrode was calculated, averaged across epochs and referenced to a baseline rest period as a percent change in power. Event related synchronization (ERS), the synchronized increase in beta band power after a cortical motor command, was attenuated ($p=0.0199$) after prolonged vibration compared to ankle dorsiflexion without vibration. Event related desynchronization (ERD), an event related decrease in spectral power found during movement initiation, was also observed to be attenuated ($p=0.0557$) after prolonged vibration. The attenuation of beta band cortical oscillations following prolonged vibration of the TA, despite no significant change in the EMG activity of the resulting ankle movement, lends evidence towards a change in cortical sensory processing during movement after proprioceptive attenuation.

Disclosures: J. Lee: None. **B.D. Schmit:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

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Title: Increased activation in premotor and prefrontal cortices relates to poorer motor action selection performance after stroke

Authors: *J. C. STEWART, S. C. CRAMER;
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Abstract: Action selection (AS) is a critical feature of voluntary movement. After stroke, damage to the motor system often leads to compensatory brain activation for the performance of simple movement tasks. It is not known, however, how the motor system responds after stroke when additional AS requirements are placed on movement and whether variability in task performance between individuals relates to neural resource utilization. The purpose of this study was to determine the relationship between AS task performance and brain activation in individuals post-stroke. Ten individuals with right-sided hemiparesis (mean (SD): age: 66.3 (5.7); Upper Extremity Fugl-Meyer motor score: 52.3 (9.1); time post-stroke: 42.5 (24.7) months) and 16 age-matched controls (age: 65.0 (9.0)) performed a right or left joystick movement with the dominant, right hand under two conditions. In the *action selection (AS) condition*, participants moved right or left based on a visual cue. In the *execution only (EO) condition*, participants repeated a simple movement in the same direction on every cue. After a practice period (3 blocks of 36 trials), the two conditions (AS, EO) were performed during functional MRI in a 3T scanner with an MRI compatible joystick. In both subject groups, reaction time (RT) for the AS condition was significantly longer than for the EO condition (p<0.001). RT was longer in the stroke group, but RT cost (AS RT normalized to EO RT) did not significantly differ between groups and did not correlate with degree of motor impairment. Across groups and tasks, movement activated a motor network that included left primary motor, PMd, supplementary motor area (SMA), and parietal cortices and right cerebellum; right PMd and parietal cortex were also active in the stroke group. Regression analysis during AS revealed three significant clusters that had a significant positive correlation with RT cost: left PMd, dorsal lateral prefrontal cortex, and SMA (p<0.001), i.e., individuals with higher cost had greater activation in these regions during AS. Interestingly, these relationships were independent of group. In summary, increased activation in left premotor and prefrontal regions during the AS task was associated with slower task performance suggesting greater utilization of these regions reflects compensatory activation to maintain task performance. The brain-behavior relationship in individuals post-stroke varied in a similar manner as controls, suggesting common compensatory mechanisms across stroke and normal aging. Future work will investigate whether neural recruitment during AS predicts motor learning in complex conditions of practice that target AS processes.

Disclosures: J.C. Stewart: None. **S.C. Cramer:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 273.07/BBB8

Topic: D.17. Voluntary Movements

Title: Neural correlates of reaching movements in virtual environments designed for neuro-rehabilitation

Authors: *G. GARIPELLI, V. LIAKONI, D. PEREZ-MARCOS, T. TADI;
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Abstract: Recent advances in neurorehabilitation research have shown that virtual reality (VR) based cognitive therapy may induce cortical plasticity and promote recovery using goal-directed arm/hand movements. Through an immersive VR setting, it may be possible for a system capable of appropriately stimulating the action observation system to encourage plasticity and repair. Since task-oriented rehabilitation is known to be beneficial, in the current study, we designed a virtual reality (VR) paradigm in combination with EEG through a novel VR platform (MindMaze, Switzerland) to investigate the behavioral and neural mechanisms associated with the observation and execution of self-generated movements in healthy participants.
64 Channel scalp EEG was recorded from 9 healthy participants (mean ± SD; 29±5 years) during a reaching task in a VR platform (MindMaze). Participants' were instructed to reach 5 equidistant targets in a virtual environment following a linear path. Their movements were recorded and mapped onto an avatar in real time. We analyzed EEG activity (Slow Cortical Potentials (SCP; 0.1-5Hz); and beta (18-30Hz) band), as well as behavioral data (reach time and trajectory accuracy) across three conditions: a) 1-to-1 mapping: right arm mapped onto the virtual right arm; b) mirror mapping: right arm mapped onto the virtual left hand; and c) video control: pre-recorded reaching movements.
Analysis of behavioral data revealed a significant main effect (Anova) of mapping with longer reach times ($F(1,8)=16.48$, $p<0.01$) and reduced trajectory accuracy ($F(4,32)=4.99$, $p<0.01$) in the mirror mapping across all the targets. Analysis of the SCP over central electrodes before movement onset showed a significant negative shift in the mirror condition compared to the other two (ANOVA, $p<0.001$). Furthermore, SCP at C1 electrode during movement correlated positively with trajectory accuracy ($\rho=0.86$ in 1-to-1; $\rho=0.48$ in mirror; Pearson coeff). We also observed higher beta activity suppression in electrodes C3, CP3 and CP1 electrodes during the movement execution in the 1 to 1 compared to the mirror condition (t-test, $p<0.001$).
Behavioral data shows that the mirror condition was more complex to perform & depending on the mapping, the same targets were accessed differently. Data from SCP suggests that the upcoming task complexity is encoded in the preparatory activity and may predict task difficulty. Additionally, weaker contralateral beta activity during the mirror mapping may be due to the increased multisensory incongruence. Further studies in patients will provide valuable insights into these manipulations signatures for concrete prognosis and diagnostics.

Disclosures: **G. Garipelli:** A. Employment/Salary (full or part-time):; MindMaze SA, Ecublens 1024, Switzerland. **V. Liakoni:** A. Employment/Salary (full or part-time):; MindMaze SA, Ecublens 1024, Switzerland. **D. Perez-Marcos:** A. Employment/Salary (full or part-time):; MindMaze SA, Ecublens 1024, Switzerland. **T. Tadi:** A. Employment/Salary (full or part-time):; MindMaze SA, Ecublens 1024, Switzerland.

Poster

273. **Cortical Motor Planning: Neuroimaging**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 273.08/BBB9

Topic: D.17. Voluntary Movements

Support: Parkinson's UK Grant

Title: The role of primary motor cortex beta activity in the control of force output

Authors: *E. PROKIC, G. WOODHALL, I. STANFORD, S. HALL;
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Abstract: Beta frequency (15-30Hz) oscillatory power in the motor network is modulated during motor control and has been linked to specific movement phases, such as movement preparation and execution. However, experimental results and opinions on the involvement of beta in force output are varied.
Excessive synchronization of neuronal activity in the beta range is characteristic of patients with Parkinson's disease (PD) and beta oscillations are thought to produce the motor impairment associated with the disease. Stimulation at 20Hz in the subthalamic nucleus (STN) and motor cortex stimulation at 20Hz has been shown to impair force development (Chen et al., 2011; Joundi et al., 2012) providing a link between excessive neuronal synchronization at beta frequencies and motor impairment in PD. However, the effects of these experiments are modest. Here, we used a source-level magnetoencephalography (MEG) approach to investigate the hypothesis that beta power suppression in primary motor cortex (M1) co-varies with motor force generation. Ten healthy participants used their dominant hand to produce specific isomentric force output, measured using a MEG compatible force transducer. Four target levels were generated (35, 50, 65 and 80% of each participants maximum force output). Participants were required to elicit the target force as quickly and accurately as possible in response to four randomly presented visual cues and maintain each force level for 5 seconds. M1 cortices were localised with synthetic aperture magnetometry beamforming analysis. Virtual electrode analysis was used to reconstruct the spontaneous and movement-related oscillatory activity in bilateral

M1 cortices, before and during force output.
We demonstrate that there is no difference in the ampitude of beta desynchronisation from pre-movement baseline at the four different force outputs. These data suggest that beta desynchronisation does not encode force output. Further experiments are underway to explore the role of beta desynchronisation in relation to force control over time, movement speed, between cue and onset of movement and between onset to target force reached.

Disclosures: E. Prokic: None. **G. Woodhall:** None. **I. Stanford:** None. **S. Hall:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

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Program#/Poster#: 273.09/BBB10

Topic: D.17. Voluntary Movements

Support: NIH Grant R01 NS065049

Title: Separable components of object-related gesture production in the left hemisphere

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Abstract: A great number of functional neuroimaging studies suggest that gesture production is associated with a widely distributed bilateral frontal, parietal, and posterior temporal network. Evidence from patients with ideomotor apraxia after left hemisphere stroke suggests, in contrast, that the left posterior temporal and inferior parietal lobes are a critical locus of gesture representations, particularly those for skilled object use. We performed voxel-based lesion symptom mapping with data from 73 left hemisphere stroke participants to assess the critical neural substrates of 3 types of gestures performed with the less-impaired left hand: gestures produced in response to viewed objects (VO), imitation of object-specific gestures demonstrated by the examiner (IO), and imitation of meaningless gestures (IM) matched to the other two types for complexity. Gestures were scored separately for postural (hand/arm positioning) and dynamic (amplitude/timing) accuracy. Lesioned voxels in the left posterior middle temporal gyrus (pMTG) were significantly associated with lower scores on the posture component for both of the object-related gesture types (VO and IO). In contrast, this region was not associated with posture scores for the meaningless gestures (IM). Moreover, lesioned voxels in the pMTG remained significant predictors of low VO and IO even when controlling for IM performance. Poor performance on the dynamic component of all 3 gesture types was significantly associated

with relatively more dorsal regions in the left inferior parietal and frontal cortices. These data confirm prior claims that the left hemisphere is a critical substrate of gestural action, and extend prior findings by demonstrating separable loci for postural and dynamic components of object-related (but not meaningless) gesture. Finally, the pMTG substrate of object-use postures suggests that these representations may have co-evolved with semantic systems encoding relational (thematic) object knowledge.

Disclosures: L.J. Buxbaum: None. **A.D. Shapiro:** None. **H.B. Coslett:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

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Topic: D.17. Voluntary Movements

Support: National Institute of Information and Communications Technology of Japan

Title: Attention modulated resting state neural activity predicts future performance on a complex multimodal glider landing task

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Abstract: The goal of this study is to determine internal states that are difficult to record behaviorally that may predict future performance. There has been considerable debate about the role of resting state brain networks in modulating task processing and performance. Most of these studies, however, utilize tasks and conditions that are far removed from real-life experience and may not translate to real-world application. In this study we utilize a neuroergonomics approach in which it is maintained that in order to investigate complex real world behavior it is necessary to understand the processes within the context of the underlying interacting brain networks rather than under reduced isolated conditions typical of brain imaging experiments that only occur in the laboratory. In accomplishing this goal an extremely robust virtual glider flight simulator is used within fMRI to investigate neural processes underlying complex perceptual, motor, and cognitive processing. Specialized MRI compatible fiber optic devices were used to duplicate the same flight controls as are used in a real glider: control stick (right hand) manipulating the aileron (roll) and elevator (pitch), rudder pedals (yaw; both feet), and dive brake (left hand). The task for the subjects (consisting of 14 pilots recruited from local university

glider clubs) was to land the glider as close to a red x on the runway as possible from a first person perspective. The fMRI results show that activity in action planning brain regions are parametrically modulated by behavioral performance on the landing task. These brain regions include the basal ganglia, primary motor cortex, premotor cortex, dorsolateral prefrontal cortex, and the parietal cortex. Of considerable interest, is the finding that in the resting condition, starting 25 seconds before each trial, these same brain regions showed an inverse parametric modulation with behavioral performance. These results suggest that modulation of brain networks on a complex task by internal states such as alertness, vigilance, attention can be determined long before the task begins. It is unlikely that these internal brain states can be measured through behavioral or physical measures that don't involve monitoring brain activity. The proposed research has far reaching implications for advancement in knowledge of brain processes involved with complex real world tasks as well as development of neuroergonomic technology that can be used in a wide variety of applications such as vehicle/machine operation, medical/psychological diagnosis, and rehabilitation/treatment.

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Poster

273. **Cortical Motor Planning: Neuroimaging**

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Topic: D.17. Voluntary Movements

Support: DFG (Deutsche Forschungs Gesellschaft) Grant HE 3592/7-1 & WO 1517/1-1

Title: Pathways of processing tool information during actual use

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Abstract: In order to use tools like a scissor, a spoon or a pen properly we need to process, connect and integrate different information in order to reach the planned goal of cutting, scooping or writing. A tool related network including parietal, frontal and temporal regions has been shown in studies investigating the imagination, pantomime or observation of tool use. The

pathway in which the semantic aspects and action relevant information is transported through this network during actual tool use and action planning is yet not fully clear. Dynamic causal modeling (DCM) is a method for the analysis of such networks in functional magnetic resonance imaging (fMRI) data with regard to changing patterns of effective connectivity among brain areas due to experimentally induced contextual modulation. The aim of this study was to define a tool use specific network during actual tool use and analyze how this network is connected and processes the tool specific information.
A special apparatus in the MRI scanner was used to present different stimuli. One set of stimuli included ten tools regularly used in daily life (e.g. scissor, spoon or pen), the other comprised ten neutral objects. The task was to either use or transport the tools and objects with the right and left hand. A signal triggered the start of the action and divided each trial in a planning and execution phase. The fMRI activation analysis revealed a tool specific activation-pattern in temporal, parietal and frontal regions representing semantic aspects, the processing of tool-knowledge and the realization of motor actions. Different models were tested to investigate how the tool related network is constructed and how the semantic information of tool use is distributed within this network.
With DCM and Bayesian model selection it was possible to detect a model of effective connectivity, which describes a network including brain regions of the dorsal and ventral stream. This network is involved in the processing of tool use specific information during the planning and the execution of an action. Additionally the analysis revealed that semantic information from the ventral stream is connected to the processes of the dorsal stream via a strong temporal parietal link.
Not only is this essential for the general knowledge about the neuronal processes of action planning and tool use but can also be relevant for certain clinical investigations. The impaired use of tools and objects can be the result of brain lesions after a stroke and is seen in patients with apraxia. In order to understand the neuroanatomical correlates of apraxia it is important to know the neuronal networks which are important for planning complex actions like tool use.

Disclosures: M. Brandi: None. **J. Hermsdörfer:** None. **C. Sorg:** None. **G. Goldenberg:** None. **A. Wohlschläger:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

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Topic: D.17. Voluntary Movements

Support: Neuroscience Center Zurich PhD Grant

Swiss National Science Foundation

Title: Improvement in precision grip control correlates with self-modulation of primary motor cortex

Authors: *M. BLEFARI[1,2], J. SULZER[2], M.-C. HEPP-REYMOND[3], S. KOLLIAS[4], R. GASSERT[2];
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Abstract: Mental imagery is a valuable tool in motor learning due to its endogenous nature, ability to complement physical training, and its proven effectiveness. It has been shown that motor imagery improves motor performance and the improvement is associated with cortical changes in primary and secondary motor areas. Yet, it is unclear how changes in each motor area represent the putative mechanisms behind motor imagery's improvement of performance.
The aim of the present study is to define the role of the primary motor cortex (M1) in motor learning by guiding mental imagery within this area using neurofeedback training. To this extent, we used real time functional magnetic resonance imaging (rtfMRI) to measure activity of the M1 hand area online and then fed this information back to the participant for the purpose of self-regulation. The M1 hand area was functionally localized in every participant during a visuomotor isometric precision grip task. Participants attempted to up-regulate the target area while performing motor imagery of pinching. Before and after M1 up-regulation, outcomes of the precision grip task were evaluated. Changes between the two assessments were analyzed to measure motor performance. We found short-term performance improvements in the isometric force-matching task to be proportional to the ability to up-regulate the BOLD signal of the target region. The endogenous self-regulation employed in this paradigm is both useful as a scientific tool to investigate the involvement of M1 in motor imagery, but also has implications towards refining mental training strategies for targeted motor improvements in motor performance.

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Poster

273. **Cortical Motor Planning: Neuroimaging**

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Topic: D.17. Voluntary Movements

Support: Institute for Collaborative Biotechnologies Grant GS1

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Title: Dissociating intrinsic and extrinsic reference frames in the human motor system

Authors: *D. A. BARANY[1], V. DELLA-MAGGIORE[2], M. CIESLAK[1], S. VISWANATHAN[1], S. T. GRAFTON[1];
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Abstract: Hand movements can be defined in relation to intrinsic and extrinsic references frames. Single-neuron recordings of movement-related activity in motor regions of monkeys have shown a mixed representation of muscle activity and posture (intrinsic), and movement direction (extrinsic). We hypothesized that intrinsic and extrinsic movement codes are differentially expressed in the human motor system. Rather than remapping reference frames using a manipulandum, we directly tested the hypothesis by exploiting two properties of movements about the wrist: (1) wrist movements involve two distinct muscle groups (ulnar-radial and extension-flexion) and (2) rotation of the forearm changes the muscle group required to produce a movement in a given direction. In a rapid event-related functional magnetic resonance imaging (fMRI) design, we measured blood oxygen level dependent (BOLD) brain activity as participants used free right-handed wrist movements of variable amplitudes to move a cursor to targets at different locations. In each run, participants maintained either a palm-down posture or palm-mid posture and moved to horizontal or vertical targets on each trial of that run. We applied multivoxel pattern analysis (MVPA) to successfully classify movements on single trials from parietal, premotor and motor areas. In each region, the classifier's generalization performance was used to dissociate target location, direction of movement, forearm posture, and muscle used. Both target location and movement direction were decodable from ventral premotor cortex (PMv). However, target location was more decodable than movement direction in the superior parietal lobule (SPL). The decoding of target location in PMv and SPL was robust to posture differences, and to targets presented in the vertical and horizontal plane. Furthermore, muscle- and posture-related information was widely decodable independent of external-space related coding, suggesting a mixed pattern of movement encoding in the human motor system.

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Poster

273. Cortical Motor Planning: Neuroimaging

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Topic: D.17. Voluntary Movements

Support: Caritro Grant ; "The effect of parietal and premotor lesions on reaching and grasping in cerebral patients" funded by Fondazione Cassa di Risparmio di Trento e Rovereto

Title: Neural dynamics of the prehension system

Authors: *L. TURELLA[1], R. TUCCIARELLI[1], R. RUMIATI[2], A. LINGNAU[1];
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Abstract: In everyday life, we continuously interact with people and objects within our social and physical environment. The most powerful and versatile tools we adopt within these complex interactions are our hands. Prehension, the capability to reach and grasp objects, is a clear example of the capacity of our hands to manipulate our environment. Even if this ability appears effortless and simple, the whole-brain network dynamics underlying its sophisticated control are still largely unknown.
Here we used magnetoencephalography (MEG) to record brain activity of nineteen subjects performing non-visually guided reaching and grasping movements on an object, using either the left or the right hand. The experimental paradigm comprised a planning and an execution phase. We observed spectral modulations induced by the experimental conditions in different frequency bands (alpha, beta) within the 'prehension' network. These modulations were not only evident within the execution phase but also during the planning phase. Changes in spectral power during the planning phase were evident both with respect to the effector used (left or right hand) and the performed action (grasping or reaching). This overview of whole-brain spectral modulations contributes towards a better understanding of the dynamics within the prehension system during the planning and execution of actions.

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Poster

273. **Cortical Motor Planning: Neuroimaging**

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Program#/Poster#: 273.15/BBB16

Topic: D.17. Voluntary Movements

Title: The EEG activity of the prefrontal cortex in the visual reaction task

Authors: *A. MORI[1], R. KOSHIZAWA[1], K. OKI[1], M. TAKAYOSE[2], Y. KITA[3];
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Abstract: Recently, we have that the frequency of electroencephalogram (EEG) component β band is synchronized with a probability of 100% in the prefrontal cortex just before the start of voluntary movement.
The EEG frequency monitoring showed that only a beta band synchronized in approximately 14 ms before the muscle discharge.
In the present study, we examined the occurrence pattern of beta band in the prefrontal cortex in the relationship between wrist extension and a ankle extension using visual stimulation task.
The subjects included seven kendo players all healthy male college students(19-21 years, mean age 20.3 years) and all right-hand. Experiments were carried was done using selected reaction assignment by visual stimuli. Using (17 inches) LCD display set up to 1.3 m in front of the subject was maintains a seated posture in the experimental easy chair in the inter-stimulus interval of ,3000-8000ms target stimulus for the standard stimulation (Target) stimulus image is the (Standard). The stimulus was presented randomly. To present a diamond as the Standard, the incidence of Target was 20%. The recording of EEG, was used (Electrical Geodesics, Inc. Ltd.) 128 channel digital EEG. The EEG recorded from the scalp for 128chs, was filtered through 3-30Hz, separated into the theta band (4-7 Hz), alpha band (8-12 Hz), beta band (13-30 Hz).
The frequency of EEG component beta band is synchronized with a probability of 100% in the prefrontal cortex in the visual reaction before the onset EMG.
The exact areas of beta activity site were shown in two locations in the hemisphere of the prefrontal cortex, as well as in a pinpoint area of the right hemisphere near the midline. We found a localized activity area in the left prefrontal area approximately 14 ms before the EMG onset of the wrist extensor muscle or ankle extensor muscle.
Our results show that in the selective reaction task, the activity of beta band was confined to the side of the left hemisphere and the frontal pole area of the prefrontal cortex with wrist extension or a ankle extension.
There was no significant difference between the wrist extension and a ankle extension for the latency period.These results suggest the possibility that there is recognition of the motor output in the motor cortex.

Disclosures: A. Mori: None. **R. Koshizawa:** None. **K. Oki:** None. **M. Takayose:** None. **Y. Kita:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

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Topic: D.17. Voluntary Movements

Support: JSPS Grant-in-Aid for Scientific Research

Title: Postural effects on cortical activity during swallowing: A fNIRS study

Authors: *M. YAMAWAKI, S. SHIBANO, E. YOKOZEKI;
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Abstract: (Backgrounds)
The swallowing center in the medulla is the key integrator of swallowing performance. There are subcortical and cortical centers above the brainstem that induced swallowing movement, however, their specific role and connections are not well understood. There are technical limitations of conventional neuroimaging techniques that require subjects to be in a supine position and/or restrict head movements. Such limitations narrow the range of experimental task options for swallowing. To challenge these issues, we applied functional near-infrared spectroscopy (fNIRS), an optical method that noninvasively measure cortical hemodynamics, for brain mapping in swallowing.
(Methods)
Eighteen of right-handed healthy male were analysed. Subjects, on the chair or in supine position were put 34-channel holder of OMM-2000 Optical Multichannel Monitor (Shimadzu, Kyoto, Japan). An increase in oxyHb is used as an indicator for brain activation. Sensorimotor cortex and frontal lobe were set as the region of the interest. Data analysis was performed according to our previous study. Optode positions were measured using a 3D magnetic space digitizer (FASTRAK-Polhemus, Colchester, VT). Probabilistic method was used to register NIRS data to MNI (Montreal Neurological Institute) standard brain space. The statistical method used was a general linear model employing a two-level summary statistics approach for random effects analysis with a one-tailed t test.
(Results)
Activation areas in each task were detected separately in SMI ($p < o.o5$ by Student t, one-tailed, FDR controlled). During swallowingin supine, activation was detected in tongue SMI and BA 40 ($p < o.o5$, one-tailed, FDR controlled). The haemodynamic pattern observed during swallowing was different in sitting versus supine position in BA 6 and BA 40 ($p < o.o5$, one-tailed, FDR controlled).
(Conclusions)

The haemodynamic pattern during swallowing appeared different in sitting versus in supine position in BA 6 and BA 40. Our findings suggest that the sensory input is more important in supine than in sitting posture. Since fNIRS measurements are limited to the cortical surface, determining cortical connections to insula and basal ganglia in swallowing requires continued research.

Disclosures: M. Yamawaki: None. **S. Shibano:** None. **E. Yokozeki:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

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Topic: D.17. Voluntary Movements

Support: JSPS KAKENHI 24800092

JSPS KAKENHI 24000012

Title: Variability of skillful motor performance is stemmed from variability of neuronal activity in wider range of brain regions recruited during motor execution

Authors: *N. MIZUGUCHI[1], S. UEHARA[1], S. HIROSE[1], S. YAMAMOTO[2], E. NAITO[1,3]; [1]Ctr. for Information and Neural Networks (CiNet), Natl. Inst. of Information and Communications Technol., Suita, Japan; [2]Grad. school of Human and Envrn. Studies, Kyoto Univ., Kyoto, Japan; [3]Grad. school of Med., Osaka Univ., Suita, Japan

Abstract: Human motor performances vary across trials even when people perform well-trained motor skills. In non-human primate study, it is shown that the variability of reaching movements is originated from the variability of spikes in the premotor cortex (Churchland et al. 2006). However, we hypothesized that variability of motor performance is also derived from variability of neuronal activity in other brain regions recruited during motor execution. In the present study, we focused on a skillful motor task and investigated brain activity that generates variability of its motor performance using functional magnetic resonance imaging (fMRI). 15 right-handed participants repeated a sequence of 5 button-presses (one sequence = ring-middle-little-index-ring) with their right hand for 10 seconds (= epoch) as fast and accurate as possible. Ten epochs were completed with inter-epoch-intervals of 12 seconds in each fMRI scan, and the participants completed 15 fMRI scans in the experiment. We calculated the number of correct sequences for each epoch as an index for the variability of motor performance, and performed parametric modulation analysis. As the participants well-trained the task before the experiment, no

consistent performance improvement was observed in the scanner. But we found that the performance (the number of correct sequences) varied across epochs. In the fMRI analysis, we found that the lower performance was associated with the higher activities in bilateral premotor cortices, dorsolateral prefrontal cortices, cingulate cortices, insular cortices and parietal cortices. Thus, the present findings suggest that variability of motor performance is stemmed not merely from variability of premotor activity but from the activity in wider range of brain regions that are recruited during motor execution.

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Poster

273. **Cortical Motor Planning: Neuroimaging**

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Topic: D.17. Voluntary Movements

Support: Caritro Grant "Impatto delle lesioni parietali e premotorie sul raggiungimento e sull'uso di oggetti in pazienti cerebrolesi"

Provincia Autonoma di Trento

Title: Hierarchical movement coding within the prehension network

Authors: *A. LINGNAU[1], R. RUMIATI[2], L. TURELLA[1];
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Abstract: Prehension is thought to consist of two main components: reaching, i.e. the transport of the hand toward an object, and grasping, i.e. the preshaping of the hand with respect to object's properties [1]. Reaching and grasping have been suggested to rely on two distinct pathways, a dorsomedial pathway, consisting of area V6A in the medial wall of the parietal cortex and the dorsal premotor cortex (PMd), and a dorsolateral pathway, consisting of anterior intraparietal sulcus (aIPS) and the ventral premotor cortex (PMv) [2-4]. Less is known, however, about the representation of hand orientation [5, 6].
Here we used multivariate pattern analysis (MVPA) of functional magnetic resonance imaging (fMRI) data to investigate the neural representations of non-visually guided prehension movements using two different grip types (precision grip, whole-hand grip) and two different hand orientations (0 degrees rotation, 90 degrees rotation). Patterns of fMRI data within parietal

and premotor nodes of the prehension system demonstrated the existence of different movement representations: bilateral aIPS and contralateral primary motor cortex were sensitive to muscular activity coding for specific prehension movements (i.e. specific combinations of grip type and hand orientation), whereas PMv seemed to be more sensitive to wrist orientation. Our results support the view of a hierarchical representation of movements within the prehension system.

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Disclosures: A. Lingnau: None. **L. Turella:** None. **R. Rumiati:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 273.19/BBB20

Topic: D.17. Voluntary Movements

Support: Israeli Center of Research Excellence (ICORE)

Human Frontiers Science Project (HFSP) Career Development Award

Sagol School of Neuroscience fellowship

Ministry of Absorption, returning citizen grant, Israel

Title: Separating the Means from the End: Differentiating Motor Actions from their intended consequences

Authors: *R. GILRON[1], A. KRASOVSKY[2], Y. YESHURUN[2], R. MUKAMEL[1];
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Abstract: To reach a certain goal, multiple actions can be executed. For example, in order to draw a circle one can perform a clockwise or counterclockwise pen trace. Thus the same goal

(drawing a circle) can be achieved by different motor actions. Here we examined the neural networks that dissociate intention from action. Using fMRI, we scanned 10 right handed subjects during a visuo-motor task. Subjects tracked a figure moving on the screen either vertically or horizontally. Tracking was performed with a cursor that was controlled by pen traces on an MR compatible digital tablet. During congruent trials (50%) the direction of cursor movement on the screen and direction of pen traces on the tablet were compatible (e.g. horizontal pen traces resulted in horizontal cursor movement). In incongruent trials the direction of pen traces and cursor movement were orthogonal (e.g. in order to move the cursor horizontally, subjects had to perform vertical pen traces). This design allowed us to dissociate the goals (directional cursor movement) from motor actions (horizontal/vertical pen traces). We used multi-voxel pattern analysis (MVPA) and a whole brain searchlight algorithm to examine brain regions discriminating the different experimental conditions. In agreement with previous findings, activity patterns in left motor, left parietal, and right motor cortices discriminated direction of pen traces regardless of cursor movement (performance accuracy of 80%, 73% and 73% respectively). Interestingly, activity patterns in the right parietal, right pre-motor, and left fronto-polar cortex discriminated sensory goals (horizontal from vertical cursor movement) regardless of pen traces (performance accuracy of 77%, 75% and 75% respectively). These results point to a network of brain regions involved in high order goal selection that is dissociated from specific motor plans.

Disclosures: R. Gilron: None. **A. Krasovsky:** None. **Y. Yeshurun:** None. **R. Mukamel:** None.

Poster

273. **Cortical Motor Planning: Neuroimaging**

Location: Halls B-H

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Topic: D.17. Voluntary Movements

Support: JSPS KAKENHI (Grant-in-Aid for Specially promoted Research 24000012)

JSPS KAKENHI (Grant-in-Aid for JSPS fellows, 25-4917).

Title: Change in resting-state functional connectivity after motor skill learning in humans

Authors: *S. UEHARA[1,2], N. MIZUGUCHI[1], S. YAMAMOTO[2,3], S. HIROSE[1,2], E. NAITO[1,4]; [1]Ctr. For Information and Neural Networks (CiNet), Natl. Inst. of Information and Communications Technol., Suita City, Osaka, Japan; [2]Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Grad. Sch. of Human and Envrn. Studies, Kyoto Univ., Kyoto, Japan; [4]Grad. Sch. of Medicine, Osaka Univ., Osaka, Japan

Abstract: Motor memories are stabilized and consolidated during resting-state after motor practice. Here, we investigated change in functional connectivity after motor practice using functional magnetic resonance imaging. Thirty-two right-handed healthy volunteers practiced two different types of sequential finger-tapping tasks with their left hands. They were subdivided into two groups; one group practiced two tasks in a randomized order (random practice; n = 16), while the other group practiced one task first and then practiced the other task next (blocked practice; n = 16). We analyzed resting-state functional connectivity before and after the motor practices.
When we analyzed the connectivity after the practices, we found that the activities between the right dorso-lateral prefrontal cortex (DLPFC) and precuneus were coupled only after random practice. As this activity coupling was not observed before either type of motor practice, the activity coupling between DLPFC and precuneus was confined after the random practice. The present findings indicate that resting-state functional connectivity after motor practice may reflect change in spontaneous brain activity associated with memory consolidation process, which is distinct between random and blocked practices.

Disclosures: S. Uehara: None. **N. Mizuguchi:** None. **S. Yamamoto:** None. **S. Hirose:** None. **E. Naito:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.01/BBB22

Topic: E.01. Neuroendocrine Processes

Support: NIH R01 Grant AG032315

Title: Ablation of arcuate KNDy neurons amplifies the LH surge in steroid-primed, ovariectomized rats

Authors: S. J. KRAJEWSKI-HALL[1], M. A. MITTELMAN-SMITH[1], N. T. MCMULLEN[2], *N. E. RANCE[1,2,3];
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Abstract: KNDy (kisspeptin, neurokinin B and dynorphin) neurons in the arcuate nucleus play an important role in the reproductive axis. We have developed a method to selectively ablate KNDy neurons in the rat using NK_3-SAP, a neurokinin 3 receptor agonist conjugated to saporin (Mittelman-Smith, Endocrinology 2012). Ablation of KNDy neurons results in cessation of

estrous cycles, ovarian atrophy, a decrease in tonic LH secretion and loss of the rise of serum LH after ovariectomy. Given these profound effects, we tested if we could induce an LH surge in KNDy-ablated rats using a well-established steroid replacement regimen. Rats were maintained on a 14:10 light cycle (lights on at 0500). Using stereotactic surgery, NK_3-SAP or Blank-SAP was injected in the arcuate nucleus and rats were allowed to recover for one month before ovariectomy. Seven days after ovariectomy they were implanted with silastic capsules containing 17β-estradiol. Two days later, they were implanted with progesterone capsules (~0830h). Rats were sacrificed in the afternoon at a time previously shown to exhibit peak LH surge levels (~1600h) and the brains were processed for immunohistochemistry. Ablation of KNDy neurons was verified by near complete loss of NKB-immunoreactive neurons in the arcuate nucleus in NK_3-SAP rats. At 0830h, tonic levels of serum LH were significantly lower in KNDy ablated rats, consistent with our previous studies. Unexpectedly, the surge in serum LH at 1600h was more than 3-fold higher in NK_3-SAP-treated rats compared to Blank-SAP controls (53.5 $\pm$ 16.5 ng/ml vs 16.5 $\pm$ 2.1 ng/ml, respectively). To determine if this change was associated with increased activation of GnRH neurons, dual-labeled GnRH-fos immunocytochemistry was performed in rostral hypothalamic sections. There was no significant difference in the total number of GnRH cells counted in 4 matched sections (NK_3-SAP, 85.8 $\pm$ 9.8 vs Blank-SAP, 84.9 $\pm$ 4.6) or in the percentage of GnRH cells that were activated, as measured by GnRH-fos coexpression (NK_3-SAP, 22.4 $\pm$ 1.8% vs Blank-SAP, 16.6 $\pm$ 4.9%). In addition, there was no difference between NK_3-SAP and Blank-SAP controls in the number of fos-ir cells counted in the AVPV. These data indicate that arcuate KNDy neurons are not required for the induction of an LH surge. The marked increase in the LH surge in KNDy-ablated rats, however, suggests that KNDy neurons are important for regulating the magnitude of the surge.

Disclosures: S.J. Krajewski-Hall: None. **M.A. Mittelman-Smith:** None. **N.T. McMullen:** None. **N.E. Rance:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.02/BBB23

Topic: E.01. Neuroendocrine Processes

Support: NIH P01 HD044232 to V.P., M.N.L., and L.M.C.

Title: Co-treatment with either insulin sensitizer or androgen antagonist blocks the effect of prenatal testosterone on AgRP cell number in female sheep hypothalamus

Authors: *M. CERNEA[1], L. M. COOLEN[1], V. PADMANABHAN[2], M. N. LEHMAN[1];
[1]Neurobio. and Anatom. Sci., Univ. of Mississippi Med. Ctr. (UMMC), Jackson, MS;
[2]Pediatrics, Obstetrics and Gynecology, and Reproductive Sci. Program, Univ. of Michigan, Ann Arbor, MI

Abstract:
Similar to women with polycystic ovarian syndrome (PCOS), prenatal testosterone (T) treated ewes develop metabolic defects such as insulin resistance and compensatory hyperinsulinemia. Previous findings from our lab have found that prenatal T-treated ewes also display alterations in hypothalamic metabolic control neurons manifested as increased numbers of immunoreactive agouti-related peptide (AgRP) neurons with no change in number of pro-opiomelanocortin (POMC) cells. In addition, we demonstrated that co-treatment of T with androgen antagonist (Flutamide, F) reversed this effect. Since AGRP cells also express insulin receptors, the current study determined whether pre- or postnatal treatment with the insulin sensitizer, Rosiglitazone (R), would reverse the effects of prenatal T on these neuronal populations. Pregnant Suffolk ewes were administered twice weekly injections of T propionate (T; 100 mg i.m.) from days 30 to 90 of gestation. In addition, separate groups of pregnant ewes received prenatal T combined with either R (8 g/ewe/day, oral) or F (15mg/kg/day, s.c.). An additional group of prenatal T-treated offspring received postnatal R (0.11 mg/kg/day, oral), beginning at 8 wk of age. Control groups received pre- or postnatal R treatments without prenatal T. At 21 months of age, all adult female offspring were ovariectomized and received hormonal implants to produce an artificial follicular phase prior to sacrifice, and brains were processed for AgRP immunocytochemistry. Confirming our previous findings, prenatal T treatment significantly increased the number of AgRP neurons ($p=0.001$), and prenatal F treatment reversed this effect, reducing cell number to control levels ($p<0.001$). Moreover, prenatal ($p<0.001$), but not postnatal ($p=0.709$), treatment with insulin sensitizer (R) reversed the effect of prenatal T on AgRP neuron number. The finding that prenatal treatments with either insulin-sensitizer or anti-androgen reverses the effect of prenatal T indicate that a common mediator involving both androgen and insulin signalling is responsible for the long-term alteration in AgRP cell number. Given the role of AgRP in regulation of energy homeostasis and body weight, we hypothesize that this effect of prenatal T on AGRP neurons may contribute to increased risk of obesity and metabolic syndrome associated with PCOS.

Disclosures: M. Cernea: None. **L.M. Coolen:** None. **V. Padmanabhan:** None. **M.N. Lehman:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.03/BBB24

Topic: E.01. Neuroendocrine Processes

Support: NIH R01 HD017864

Title: Neurokinin B signaling in the retrochiasmatic area is essential for the full preovulatory LH surge in ewes

Authors: K. L. PORTER, S. M. HILEMAN, S. L. HARDY, *R. L. GOODMAN;
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Abstract: The effects of inactivating mutations indicate that neurokinin B (NKB) is essential to human reproduction, but its physiological roles in control of GnRH secretion remain unclear. Recently, we observed that senktide, an NKB receptor (NK3R) agonist, stimulates surge-like LH secretion in ewes when given in the retrochiasmatic area (RCh) or preoptic area (POA). Thus NKB may play a role in E2 positive feedback to induce the preovulatory LH surge. The aims of this study were to determine: 1) if RCh or POA senktide treatment activates kisspeptin neurons in the arcuate nucleus (ARC), and 2) if NKB-NK3R signaling in the RCh or POA is necessary for the preovulatory LH surge. For Exp. 1, bilateral senktide-containing or empty microimplants (n=3/treatment group) were placed in the RCh or POA in anestrous ewes. Ewes were killed and brains perfused 3 h later, tissue collected, and sections encompassing the ARC used for dual immunocytochemistry to assess the percentage of kisspeptin-ir neurons that were activated, based on coexpression of cFos. In prior crossover experiments, senktide stimulated surge-like LH secretion in all 6 RCh and 5/6 POA ewes. cFos in ARC kisspeptin neurons was induced by senktide in the RCh (59.0 ± 8.6% vs. 4.0 ± 0.5% in controls, $P < 0.05$) and, albeit to a lesser extent, in the POA (26.8 ± 5.0% vs. 4.8 ± 1.1%, $P < 0.05$). In Exp. 2, ewes were OVX and chronic guide tubes were placed in the RCh or POA. At the time of surgery, a 1 cm E2-containing silastic implant and progesterone-containing controlled internal drug-releasing devices (CIDRs) were inserted sc and intravaginally, respectively. After 10-14 days, CIDRs were removed and four 3 cm E2 implants were inserted 24 h later. Microimplants containing SB222200, an NK3R antagonist, or empty control microimplants , were immediately inserted in the RCh or POA (n=5/group). Jugular blood samples were collected every 2 h for a period of 48 h. Microimplants and the longer E2 implants were removed, CIDRs reinserted and the protocol repeated in a cross-over design. Microimplants of SB222200 in the RCh significantly decreased the amplitude of the LH surge from 185.2 ± 10.2 ng/ml with empty implants to 108.4 ± 17.5 ng/ml. In contrast, SB222200 treatment in the POA had no effect on LH surge amplitude (79.7 ± 15.2 vs. 78.3 ± 17.4 ng/ml, control vs. SB222200, respectively). No effects on timing of the LH surge were observed. Together with tract tracing data (Coolen et al., SFN abstr 2013), these findings indicate that NKB signaling in the RCh, but not the POA, is essential for the full preovulatory LH surge, and that the effect of NKB in the RCh on the LH surge is likely mediated, at least in part, by direct projections to ARC kisspeptin neurons.

Disclosures: K.L. Porter: None. **R.L. Goodman:** None. **S.M. HIleman:** None. **S.L. Hardy:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.04/BBB25

Topic: E.01. Neuroendocrine Processes

Support: HD017864

NIFA 2011-67015-30059

CRIS 5432-31000-091-00D

Title: Effect of progesterone on kisspeptin and neurokinin B cell numbers in the arcuate nucleus of the female pig

Authors: S. M. HILEMAN[1], L. J. MEADOWS[1], K. L. PORTER[1], *L. M. COOLEN[2], C. FERGANI[2], L. A. REMPEL[3], R. A. CUSHMAN[3], W. T. OLIVER[3], E. C. WRIGHT[3], J. R. MILES[3], C. A. LENTS[3];
[1]Physiol. and Pharmacol., West Virginia Univ., Morgantown, WV; [2]Physiol. & Biophysics, Univ. of Mississippi Med. Ctr., Jackson, MS; [3]USDA, ARS, U.S. Meat Animal Res. Ctr., Clay Center, NE

Abstract: Progesterone acts at the hypothalamus to inhibit LH secretion in the pig, but the mechanism for this is unknown. Kisspeptin and neurokinin B (NKB) have both been shown to influence GnRH/LH secretion and mediate steroid negative feedback in several species and to be critical for normal reproductive function in humans. This study examines the influence of progesterone on kisspeptin and NKB expression in the pig and their potential colocalization in neurons of the arcuate nucleus of the hypothalamus (ARC). The hypothesis was tested that progesterone would reduce LH secretion in association with a decrease in ARC kisspeptin or NKB cell number. White crossbred, postpubertal gilts (106 ± 1.5 kg) were surgically fitted with an indwelling jugular catheter and either ovariectomized (OVX; n=3) or ovariectomized and treated with progestin (Altrenogest, 15 mg/d; OVX+P; n=4) for 10 days beginning 2 to 3 weeks after OVX. Blood samples were collected at 12 min intervals for 4.6 hours on day 9 of treatment. On day 10 of treatment, gilts were euthanized and brain tissue collected for immunocytochemical analysis of kisspeptin and NKB expression across five or three sections, respectively, of the caudal ARC. Progesterone treatment reduced ($p<0.05$) mean LH (OVX, 1.58+/-0.20 ng/ml;

OVX+P, 0.70+/-0.23 ng/ml) and increased ($p<0.05$) LH interpulse interval (OVX, 43.5+/-5.1 min; OVX+P, 184.5+/-44.7 min) without significantly affecting LH pulse amplitude. Contrary to our hypothesis, kisspeptin cell numbers were increased ($p<0.05$) in progesterone-treated gilts (OVX, 334.7+/-26.5 cells; OVX+P, 446.5+/-47.5 cells). A similar trend was noted for NKB cell numbers, but this difference was not significant ($p>0.20$). Dual immunofluorescence revealed that kisspeptin and NKB were highly colocalized in the ARC. These data indicate that reduction of LH secretion by progesterone in the pig does not involve a decrease in kisspeptin or NKB expression. The USDA is an equal opportunity provider and employer.

Disclosures: S.M. Hileman: None. **C. Fergani:** None. **L.M. Coolen:** None. **L.J. Meadows:** None. **K.L. Porter:** None. **C.A. Lents:** None. **L.A. Rempel:** None. **J.R. Miles:** None. **R.A. Cushman:** None. **W.T. Oliver:** None. **E.C. Wright:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.05/BBB26

Topic: E.01. Neuroendocrine Processes

Support: P01 HD44232

NIH R01 ES16541

Title: Prenatal testosterone alters KNDy cell peptides in the arcuate nucleus of pre-pubertal male and female lambs

Authors: *C. FERGANI[1], L. M. COOLEN[2], V. PADMANABHAN[3], M. N. LEHMAN[1]; [1]Neurobio. and Anatom. Sci., Univ. of Mississippi, Jackson, MS; [2]Physiol., Univ. of Mississippi Med. Ctr., Jackson, MS; [3]Obstetrics and Gynecology,Pediatrics and Reproductive Sci. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: Female sheep exposed to excess testosterone (T) during prenatal life, display an array of symptoms similar to those observed in women with PCOS. Such perturbations include early onset of puberty followed by disruption of estrous cyclicity and impaired steroid feedback mechanisms in adulthood. These deficits are associated with an imbalance in Kisspeptin-Neurokinin B (NKB)-Dynorphin (KNDy) neuropeptides in the ARC nucleus of the hypothalamus of adult ewes. However, it is unknown if the alterations in KNDy peptides precede and potentially contribute towards prenatal T induced pubertal advancement. We tested the hypothesis that prenatal T treatment would alter the expression of KNDy peptides in prepubertal

females to the magnitude seen in control males. Pregnant Suffolk ewes were given injections of T propionate (100mg/kg i.m. twice weekly) from day 60 to 90 of gestation and their male and female offspring together with a group of offspring from control pregnant ewes, (n=5/group) were perfused at 10 weeks of age. Hypothalamic sections were processed for kisspeptin, NKB, and dynorphin, and numbers of immunopositive cells counted in the ARC. Sex differences in kisspeptin and NKB, but not dynorphin, were observed in control lambs, with greater numbers of kisspeptin (189 ± 13 vs. 125 ± 30, mean±SEM) and NKB (108 ± 20 vs. 62 ± 11, mean±SEM) cells in females compared to males. Prenatal T treatment significantly reduced the number of kisspeptin-positive cells in female lambs (from 189 ± 13 to 122 ± 23, mean±SEM) but had no effect on males. By contrast, prenatal T treatment increased the number of NKB cells in both females (108 ± 20 to 180 ± 17, mean±SEM) and males (from 62 ± 11 to 126 ± 21, mean±SEM). Dynorphin cell numbers were low, but prenatal T decreased the number of dynorphin-positive cells (from 12 ± 1 to 4 ± 1, in both sexes, mean±SEM). The results of the present study provide evidence that prenatal T treatment causes a masculinization of the female kisspeptin system prepubertally whereas the early onset of puberty in female lambs is accompanied by an increase in NKB and a decrease in dynorphin. Given the role of NKB in puberty, we speculate that KNDy peptide imbalance, consisting of increased stimulatory NKB and decreased inhibitory dynorphin may contribute to the early onset of puberty that occurs as a consequence of prenatal T excess.

Disclosures: C. Fergani: None. **M.N. Lehman:** None. **L.M. Coolen:** None. **V. Padmanabhan:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.06/CCC1

Topic: E.01. Neuroendocrine Processes

Support: NIH R01 HD17864

Title: Arcuate KNDy neurons receive afferent projections from the retrochiasmatic area in the ewe

Authors: L. M. COOLEN[1], T. G. SMITH[1], *M. N. LEHMAN[2], S. M. HILEMAN[3], J. M. CONNORS[3], R. L. GOODMAN[3];
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Abstract: Gonadotropin-releasing hormone (GnRH) secretion is controlled by steroid hormones via negative and positive feedback actions on interneurons. One critical population for steroid feedback action in the ovine brain is a group of arcuate (ARC) neurons that co-express kisspeptin, neurokinin B (NKB), and dynorphin: KNDy neurons. Several groups have proposed that within this cell group, kisspeptin is the stimulatory output to GnRH neurons; NKB is stimulatory to other KNDy cells, while dynorphin inhibits KNDy activity. However, we recently demonstrated that NKB may also act in the retrochiasmatic area of the hypothalamus (RCh) to regulate LH secretion. RCh neurons express NK3 receptors (NK3R) and microimplants of a NK3R agonist produces a surge-like increase in LH. The pathways by which RCh neurons ultimately control GnRH and LH release are unclear. Here, we test the hypothesis that RCh neurons have direct projections to KNDy neurons. To address this, the anterograde tracer, biotinylated dextrane amine (BDA), was injected into the RCh of ewes during anestrus (n=3). Ten days after injection (to allow for transport) ewes were perfused intracranially with 4% paraformaldehyde and brains were sectioned. Dual immunoperoxidase and fluorescence techniques were used to visualize BDA and kisspeptin. All three ewes had BDA injections restricted to the RCh, but the spread of the BDA infusion within the RCh varied and one animal had very limited labeling of cell bodies. In all animals, BDA-labeled axon terminals could be traced to the ARC, ipsilateral to the injection site, and were found in close apposition with kisspeptin cells. Moreover, a large portion (70-80%) of ARC kisspeptin cells received RCh inputs and often more than one input was noted. These results demonstrate that KNDy neurons receive direct input from neurons in the RCh. Consistent with this observation, administration of the NK3R agonist senktide into the RCh induces cFos expression in KNDy neurons (Porter et al, SFN abstract 2013). It remains to be determined whether the inputs to KNDy cells derive from NK3R-expressing or other neurons in the RCh. Moreover, we are currently testing whether connections between RCh and KNDy cells may be reciprocal.

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Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.07/CCC2

Topic: E.01. Neuroendocrine Processes

Support: K99 HD071970

R21 DH066495

U54 HD 028138

Title: Interactions between substance p, neurokinin a and kisspeptin in the control of gnrh release

Authors: *V. M. NAVARRO[1], S. SIMAVLI[1], M. WU[2], R. S. CARROLL[1], M. ALREJA[2], U. B. KAISER[1];
[1]Med., Brigham and Women's Hosp. / Harvard Med. Sch., Boston, MA; [2]Psychiatry, Yale Univ., New Haven, CT

Abstract: Kisspeptins (encoded by Kiss1) are the most potent elicitors of GnRH identified to date; yet, what regulates kisspeptin release is only starting to be deciphered. Recent studies showed co-localization of kisspeptin with neurokinin B (NKB) and dynorphin in neurons of the hypothalamic arcuate nucleus (ARC) in rodents, so-called KNDy neurons. Importantly, NKB belongs to the tachykinin family of neuropeptides that also includes substance P (SP) and neurokinin A (NKA). Studies in rodents, sheep and monkeys have documented a kisspeptin-dependent stimulatory action of NKB on gonadotropin release under physiological levels of sex steroids. However, the reproductive roles, if any, of SP and NKA remain unknown. In this work, we aimed to characterize and compare the actions of SP, NKA and NKB on gonadotropin release by using specific agonists for their corresponding receptors - SP: neurokinin receptor 1 (NK1R, GR73632); NKA: NK2R (GR64349) and NKB: NK3R (senktide) - through a series of functional and electrophysiological studies in mice. Central administration of NK1R and NK2R agonists (600 pmol) resulted in an increase in serum LH in adult intact males and adult ovariectomized and estradiol-replaced (OVX+E2) females 25 min after treatment, similar to the previously reported action of senktide. Interestingly, in the absence of circulating estradiol (OVX+sham), central injection of NK2R agonists_but not NK1R agonists_replicated the inhibitory action of senktide on LH release in this model. Additionally, we assessed the regulation of Tac1 (encoding SP and NKA) by sex steroids in the mouse brain. Tac1 mRNA was widely detected in the brain and was significantly inhibited by E2 in the arcuate nucleus (ARC), but did not co-localize with Kiss1. In order to assess a likely direct action of SP and NKA on kisspeptin release, we studied the electrophysiological effects of tachykinin receptor agonists (1-10 μM, 15-30 s) using whole-cell recordings of Kiss1-GFP neurons. All 3 agonists activated Kiss1-GFP neurons. NK1 and NK2 agonists activated 37% (n=33) and 15% (n=20) of KNDy neurons, respectively. Importantly, GnRH-GFP neurons remained unresponsive to the same challenge, suggesting a specific effect on Kiss1 neurons. In sum, we document the ability of all three members of the tachykinin family to regulate gonadotropin release through, at least in part, a stimulatory action on upstream KNDy neurons, in line with previous reports of NKB action.

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Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.08/CCC3

Topic: E.01. Neuroendocrine Processes

Support: NSF IOS-1025893

Title: Investigating circadian regulation of Rfrp (GnIH) neurons in male and female mice

Authors: *M. C. POLING, E. Y. LUO, A. S. KAUFFMAN;
Reproductive Med., UC San Diego, La Jolla, CA

Abstract: RFamide-related peptide 3 (RFRP-3, encoded by *Rfrp*) is the mammalian ortholog to gonadotropin-inhibiting hormone (GnIH) and thought to be a negative regulator of the reproductive axis due to its inhibitory effects on GnRH neurons and LH release. However, the regulatory roles that endogenous RFRP-3 plays in reproductive physiology are unclear. In rodents, RFRP-3 neurons receive projections from the suprachiasmatic nucleus (the circadian clock), and both *Rfrp* gene expression and *Rfrp* neuronal activation are reduced during the circadian-timed preovulatory luteinizing hormone (LH) surge in female hamsters. However, similar experiments have not yet been performed in mice. Nor is it known if the circadian changes in *Rfrp* expression and neuronal activation seen during the LH surge are specific to this neuroendocrine event or represent a normal daily fluctuation of RFRP-3. We therefore examined *Rfrp* expression and neuronal activation of *Rfrp* neurons in LH surge and non-LH surge paradigms in female and male mice. First, we used *in-situ* hybridization (ISH) to evaluate possible circadian changes in *Rfrp* expression in adult male mice over the course of day. There were no significant temporal changes in the number of detectable *Rfrp* neurons or the amount of *Rfrp* mRNA produced in each cell. Next, we performed double label ISH for *cfos* mRNA induction in *Rfrp* neurons of these same male mice, but observed no significant changes in the number of *Rfrp* neurons co-expressing *cfos* across time points. Nearly 50% of all *Rfrp* neurons co-expresses *cfos*, regardless of time of day, indicating a high level of *Rfrp* neuronal activation throughout the day and suggesting that *Rfrp* neurons are not circadian-gated in adult male mice. However, this does not exclude the possibility of circadian regulation of RFRP-3 neurons in females during the LH surge. To address this, we gonadectomized and estradiol-replaced female and male mice to generate an evening LH surge in females (male mice do not produce an LH surge, even with estradiol treatment). We are currently analyzing the brains of these mice to determine if *Rfrp* expression and/or neuronal activation decreases during the LH surge in female mice, as has been reported previously in hamsters. Male mice will also be examined to see if similar temporal changes occur under these conditions, or if any observed circadian decrease in

Rfrp expression or neuron activation is a sexually dimorphic feature. Overall, these findings will provide further insight into possible roles and regulation of RFRP-3 in the murine brain.

Disclosures: M.C. Poling: None. **E.Y. Luo:** None. **A.S. Kauffman:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.09/CCC4

Topic: E.01. Neuroendocrine Processes

Support: U54 HD012303

R01 HD072754

R01 HD020377

R01 HD065856

Title: Vax1 is required for migration of hypothalamic GnRH neurons and normal reproductive function in mice

Authors: *H. M. HOFFMANN[1], I. KIMURA[1,2], A. TAMRAZIAN[1], A. S. KAUFFMAN[1], P. L. MELLON[1];
[1]Dep. of Reproductive Med., UC San Diego, La Jolla, CA; [2]Kyoto Univ. Grad. Sch. of Pharmaceut. Sci., Kyoto, Japan

Abstract: Sexual maturation and fertility are regulated by a small population of neuroendocrine cells localized in the hypothalamus, the gonadotropin-releasing hormone (GnRH) neurons. In humans, disruption of the GnRH system leads to reduced fertility or complete infertility owing to hypogonadotropic hypogonadism. GnRH neurons originate in the olfactory placode at embryonic day 11.5 (e11.5), from where they
initiate their migration through the cribriform plate to the hypothalamus. Any alteration in GnRH neuron migration, survival or maturation is prone to result in hypogonadotropic hypogonadism. As a result, biological factors regulating these cellular functions are of critical interest for the treatment of GnRH-associated fertility disorders. GnRH neuron development is dependent on the sequential activation of an array of homeobox genes. Alteration of their expression causes cell death, incorrect localization, incomplete maturation and/or faulty functioning of these neurons. Here, we identified the homeodomain
transcription factor Ventral anterior homeobox1 (Vax1) as a critical regulator of GnRH neuron

function in mice. $Vax1^{-/-}$ mice had normal GnRH neuron numbers at e13.5, whereas no GnRH neurons were detected at e17.5 in $Vax1^{-/-}$. Interestingly, $Vax1^{+/-}$ retain 50% of this neuronal population at e17.5. Since $Vax1^{-/-}$ mice die soon after birth; we conducted a fertility study in $Vax1^{+/-}$ mice. $Vax1^{+/-}$ male mice fathered smaller litters than wild-type males. Analysis of sperm quality revealed that the sub-fertility was attributed to an 88% reduction of motile sperm. $Vax1^{+/-}$ females mothered smaller and less litters in 3 months than wild type. In addition, they had prolonged estrus cycles, spent more time in metestrus, and had a 60% reduction in corpora lutea number. They also exhibited increased levels of luteinizing hormone and estradiol. Surprising, despite the reduced number of GnRH neurons, we found that adult $Vax1^{+/-}$ mice had normal or slightly increased GnRH transcription levels in the hypothalamus, suggesting an important role for Vax1 in regulating GnRH transcription in vivo. To evaluate the transcriptional role of Vax1 in GnRH neurons we transiently transfected the mature GnRH cell line GT1-7 with Vax1 siRNA. qRT-PCR revealed Vax1 to be a repressor of GnRH transcription, supporting the increased transcriptional levels of GnRH found in $Vax1^{+/-}$ mice. In conclusion, reduced expression of Vax1 is associated with sub-fertility in adulthood of both male and female mice. We hypothesize that mutations in one Vax1 allele are sufficient to affect fertility and are likely responsible for cases of sub-fertility in humans.

Disclosures: H.M. Hoffmann: None. **I. Kimura:** None. **A. Tamrazian:** None. **A.S. Kauffman:** None. **P.L. Mellon:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.10/CCC5

Topic: E.01. Neuroendocrine Processes

Support: NIH U54 HD012303

R01 DK044838

R01 HD072754

T32 HD007203

Title: Neurokinin B induces c-fos transcription via serum response factor (SRF) and signal transducer and activator of transcription (STAT) in gonadotropin-releasing hormone neurons

Authors: *C. A. GLIDEWELL-KENNEY, P. P. SHAO, P. L. MELLON;
Reproductive Med., Univ. of California San Diego, LA JOLLA, CA

Abstract: A small population of hypothalamic neurons secretes gonadotropin-releasing hormone (GnRH), the hormone responsible for puberty and reproduction. GnRH stimulates the secretion of the pituitary gonadotropin hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH) to control reproduction. Mutations in Neurokinin B (NKB) and its receptor, the Neurokinin 3 receptor (NK3R), were identified in patients with hypogonadotropic hypogonadism, a disorder characterized by lack of puberty and infertility. Treatment with exogenous GnRH restored hormone levels and fertility, indicating that NKB regulates reproduction via a hypothalamic mechanism. Senktide, an NK3R agonist, given by intracerebroventricular (i.c.v) injection into rodents induces c-fos mRNA, a marker of neuronal activation, in kisspeptin neurons by in situ hybridization. In addition, NK3R is expressed in a subset of GnRH neurons, suggesting there is also a potential for NKB to directly regulate GnRH secretion. Previously, we showed that NKB induces c-fos mRNA and GnRH secretion in an in vitro model of the GnRH neuron (GT1-7 cells). Here, we elucidate the signal transduction pathways activated downstream from NK3R in GnRH neurons. Using truncation analysis of a well-characterized murine c-fos promoter, we find that the induction of c-fos gene transcription by senktide maps to between 400 and -200 bp upstream from the transcription start site. Using cis-mutations within this region, we find that the STAT (-345) and SRF (-310) binding sites are required for induction in addition to a potential modulatory role for the Ets site (-318). Using a multimer reporter and inhibitors, we show that the SRF site is sufficient for induction and utilizes a PKC-dependent mechanism. Furthermore, PKC activation leads to decreased binding of phosphorylated SRF to the -310 site and increased binding of phosphorylated Elk-1, its accessory protein, to the -318 Ets site by electromobility shift assay. In conclusion, NKB may induce c-fos transcription in GnRH neurons by a PKC-dependent mechanism involving changes in the binding of phosphorylated SRF and Elk-1 to the c-fos promoter.

Disclosures: C.A. Glidewell-Kenney: None. **P.P. Shao:** None. **P.L. Mellon:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.11/CCC6

Topic: E.01. Neuroendocrine Processes

Support: NICHD/NIH GRANT U54 HD058155

Title: Vasoactive intestinal peptide modulation of steroid-induced lh surge involves kisspeptin signaling in young but not in middle-aged female rats

Authors: *Y. SUN[1], A. KAUFFMAN[2], J. KIM[2], A. R. KHAN[2], J. SHU[1], G. NEAL-PERRY[1]; [1]Ob/Gyn, Albert Einstein Col. of Med., New York, NY; [2]Dept. of Reproductive Med., Univ. of California, San Diego, CA

Abstract: Female reproductive aging is characterized by a delayed and attenuated LH surge. We previously reported age-related LH surge dysfunction reflects reduced input from kisspeptin. Vasoactive intestinal peptide (VIP) neurons located in SCN excite GnRH cells and affect the LH surge. Of note, age-related LH surge dysfunction is characterized by reduced SCN VIP transcript. The SCN receives inhibitory input from RFamide-related peptide-3 (RFRP-3) cells in the DMN. VIP neurons project to GnRH neurons and to AVPV neurons, raising the possibility that VIP neurons may regulate GnRH neurons through effects on Kiss1 cells located in the AVPV or from RFRP-3 input. *We hypothesize RFRP-3 regulates VIP and VIP regulates GnRH and AVPV Kiss1 cell activation in young (Y) and middle-aged (MA) females.* Awake and freely moving Y (3-4 mon) and MA (9-11 mon) SD rats (n=14) with a 3rd ventricle cannula were infused with VIP (6 nmol) or saline (control) on the day of LH surge between 1300 and 1600h. Rats were killed between1600 to 1700h, brains collected and sectioned for double-label *ISH* for *GnRH/cfos*, *Kiss1*/*cfos*, and *Rfrp/cfos*. 2-way ANOVA determined group differences. $P<0.05$ = significant. *Post hoc* tests = Bonferroni or *Turkey's* test. **Results:** 55% *GnRH* cells in preoptic area (POA) and 39% *Kiss1* cells in AVPV express *cfos* in E+P primed, control Y rats. Compared to Y controls, VIP infusion reduced the percent of *GnRH* (30%) and *Kiss1* (22%) cells expressing *cfos* ($P<0.01$). The percent of *GnRH* (30%) and *Kiss1* (20%) cells expressing *cfos* was reduced in control MA rats ($P<0.01$). MA control rats also had less *Kiss1* mRNA per cell compared with Y controls ($P<0.01$). Unlike Y rats, VIP infusion in MA females increased the percent *GnRH/cfos* neurons (45%) ($P<0.01$). In contrast, VIP infusion did not affect the percent of *Kiss/cfos* cells or *Kiss1* mRNA content per cell in MA rats ($P=0.08$). VIP infusion did not affect *GnRH* or *Kiss1* cell density in Y or MA rats. *Rfrp/cfos* (9%) was not affected by age or VIP treatment. **Conclusion:** Reproductive age affects many E-regulated neurotransmission. We report VIP reduces the percent of *GnRH/cfos* and *Kiss1*/*cfos* cells in Y, suggesting that VIP-induced effects on Kiss1 cells disrupts GnRH cells. VIP increased the percent of *GnRH/cfos* but not *Kiss1/cfos* cells in MA, suggesting a direct effect on GnRH cells or other non-kisspeptin circuits that modulate GnRH cell activation. Age-related differences in VIP's effects on *Kiss1* neurons suggests that changes in GnRH neuron activation may also reflect, in part, an age-related decline in *Kiss1* neuron responsiveness to VIP.

Disclosures: Y. Sun: None. **A. Kauffman:** None. **J. Kim:** None. **A.R. Khan:** None. **J. Shu:** None. **G. Neal-Perry:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.12/CCC7

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant RO1 NS35467

Title: Kisspeptin antibody crossreactivity with GnIH: Further questions about the identity of kisspeptin in birds

Authors: *F. N. MADISON, G. F. BALL;
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Abstract: Kisspeptin (KP; product of Kiss1 gene) and its receptor (GPR54) have been shown to play a critical role in the regulation of reproduction in many vertebrate species. It has been shown for example that KP directly innervates and stimulates gonadotropin releasing hormone (GnRH) neurons that in turn stimulate luteinizing hormone (LH) release from the pituitary in mammals. Over the past several years, substantial evidence has accumulated establishing KP's role in the neuroendocrine regulation of reproduction in mammals. Genomic methods have identified KP in many non-mammlian species, but curiously not in birds. We investigated the possible presence of KP in songbirds and quail by employing an antibody that had previously been successfully used to label kisspeptin-like-immunoreactivity (KP-ir) in the medial preoptic area of a seasonal breeding avian species, the Mallard drake. The purpose of this study was therefore to characterize the distribution of KP-ir in several avian species. The brains of two female zebra finches, canaries, and Japanese quail were extracted and post fixed in acrolein. The brains were sectioned and stained via immunocytochemistry and labeled for KP using a commercial polyclonal antibody raised against the human kisspeptin-10 peptide. We found dense KP-ir fibers and perikarya in the paraventricular nucleus (PVN) and the preoptic area (POA) in all three species in addition to KP-ir fibers in the median eminence. However, while investigating the specificity of the KP antibody in tissue from all three species, we ran a control by preabsorbing the primary antibody at a 10-fold molar excess (relative to our IgG concentration) of either the KP or gonadotropin-inhibiting hormone (GnIH) antigen. GnIH is a neuropeptide (discovered in Japanese quail) that is expressed in cell bodies in the PVN and can inhibit luteinizing hormone release. GnIH-ir fibers project to GnRH neurons in the POA and to the median eminence. Interestingly, preabsorbing the KP antibody with KP and GnIH eliminated all KP-ir in both the PVN and POA in the songbird and quail tissue. Our studies suggest that this KP antibody derived from mammalian KP is detecting GnIH in birds. This finding is consistent

with the questions raised by other studies employing other methods as to whether KP is present in avian species. It is interesting to note that central injections of KP have been shown to stimulate the release of LH in the Mallard drake, suggesting that the structure of mammalian KP may share some properties in common with an unidentified novel neuropeptide responsible for modulating GnRH secretion in birds.

Disclosures: F.N. Madison: None. **G.F. Ball:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.13/CCC8

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant NS43330

NIH Grant DK68098

Title: 17β-Estradiol regulation of the mRNA expression of tachykinins in the guinea pig hypothalamus during positive feedback

Authors: *M. A. BOSCH[1], Y. FANG[1], M. J. KELLY[1,2], O. K. RONNEKLEIV[1,2];
[1]Dept. Physiol. & Pharmacol., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Oregon Natl. Primate Res. Ctr, Div. of Neurosci., Oregon Hlth. & Sci. Univ., Beaverton, OR

Abstract: Kisspeptin and neurokinin B (NKB) are essential for reproductive function. Similar to these peptides, substance P (SP)-expressing neurons are also localized in the mediobasal hypothalamus and believed to be involved in the control of reproductive functions. The NKB receptor, tac3, is expressed in Kiss1 neurons and its mRNA is reduced following E2-treatment, at least in rodent models. The guinea pig, similarly to primates, has a long ovulatory cycle with an estrogen-dominated follicular phase and a progesterone-dominated luteal phase and is therefore an excellent model for studying E2 regulation of positive and negative feedback. Although, the distribution and E2-regulation of Kiss1 neurons have been explored in GP (Bosch et al., J. Comp Neurol. 2011), the role or E2-regulation of the tachykinins and their receptors have not been investigated. Using cell harvesting of arcuate neurons and single cell (sc) RT-PCR we determined that NKB was expressed in approximately 63% of Kiss1-identified neurons, and the tac1 receptor was co-localized in 50% of the neurons. We have found previously that immunoreactive SP neurons are highly localized in the arcuate nucleus (Rønnekleiv et al., J. Comp. Neurol., 1984). For a quantitative analysis of E2 regulation of mRNA expression, we

used real-time quantitative (q)PCR of RNA extracted from micro-dissected periventricular (pv)POA and arcuate tissues in ovariectomized (OVX) oil- and E2-treated GP. This analysis revealed that NKB mRNA expression was significantly reduced ($p<0.01$; n=6) in the arcuate nucleus, but not in the pvPOA in E2-treated animals. Similarly to that found in the rodent for the tac3 receptor, the tac1 receptor was significantly reduced ($p<0.0005$; n=6) in the arcuate nucleus in E2 compared to oil-treated GP. Interestingly, the tac1 receptor mRNA levels were increased ($p<0.05$; n=5-6) in the pvPOA in E2-treated GP. These findings suggest a significant role for both NKB and SP systems in mediating E2-feedback to the hypothalamic areas involved in the control of reproduction and other homeostatic functions.

Disclosures: M.A. Bosch: None. **Y. Fang:** None. **M.J. Kelly:** None. **O.K. Ronnekleiv:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

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Program#/Poster#: 274.14/CCC9

Topic: E.01. Neuroendocrine Processes

Support: NS38809(MJK)

NS43330 (OKR)

DK68098 (MJK & OKR)

HD049651 (RAS)

Title: Conductances underlying the burst firing of kisspeptin neurons in the rostral periventricular area of the third ventricle (RP3V)

Authors: *C. ZHANG[1], K. J. TONSFELDT[1], J. QIU[1], R. A. STEINER[2,3], M. J. KELLY[1,4], O. K. RØNNEKLEIV[1,4];
[1]Dept Physiol & Pharmacol, Oregon Hlth. & Sci. Univ., PORTLAND, OR; [2]Dept Obstetrics & Gynecology, Univ. of Washington, Seatle, WA; [3]Dept Physiol & Pharmacol, Univ. of Washington, Seatle, WA; [4]Div. of Neurosci., Oregon Natl. Primate Res. Ctr., Portland, OR

Abstract: Kisspeptin (Kiss1) neurons in the rostral periventricular area of the third ventricle (RP3V) provide excitatory drive to gonadotropin-releasing hormone (GnRH) neurons to control fertility. Using whole-cell patch clamp recording and scRT-PCR techniques targeting Kiss1-CreGFP neurons, we characterized the biophysical properties of these neurons and identified the critical molecular constituents required for burst firing in estrogen-treated female mice.

Approximately 25% of the neurons exhibited burst firing, but all Kiss1 neurons in this region expressed a kinetically fast pacemaker current, (I_h) with mean amplitude of 31.3 ± 4.8 pA. Single-cell PCR analysis of Kiss1- neurons revealed abundant expression of the HCN1 channel transcript, which was 3-fold greater than either HCN2 or 3. The rapidly inactivating T-type Ca^{2+} current was prominent in these neurons, with a mean amplitude of 41.5 ± 5.8 pA, and molecular analysis revealed that $Ca_V3.1$ mRNA was highly expressed in these cells. Interestingly, Kiss1 neurons in RP3V have much higher (2.4 GΩ) input resistance in comparison to Arc Kiss1 neurons (0.65 GΩ), which lowers the rheobase for generating burst firing. Kiss1 neurons also expressed a persistent sodium current with a variable amplitude and activation threshold. We found that T-type calcium channels plays a critical role in driving the low threshold burst firing, but the h-current and the persistent sodium current also help drive burst firing. Therefore, the interaction of multiple conductances facilitates the burst firing of Kiss1 neurons in the RP3V.

Disclosures: C. Zhang: None. **K.J. Tonsfeldt:** None. **J. Qiu:** None. **R.A. Steiner:** None. **M.J. Kelly:** None. **O.K. Rønnekleiv:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.15/CCC10

Topic: E.01. Neuroendocrine Processes

Title: Changes in RFamide related peptide-1 (RFRP-1)-immunoreactivity during postnatal development and estrous cycle

Authors: *M. D. ANDERSEN, S. R. JØRGENSEN, A. OVERGAARD, J. D. MIKKELSEN; Neurobio. Res. Unit, Rigshospitalet, Copenhagen, Denmark

Abstract: Gonadotropin releasing hormone (GnRH) is a key player in the hypothalamic control of gonadotropin secretion from the anterior pituitary gland. GnRH secretion is governed by a dynamic interplay between excitatory and inhibitory signals to GnRH neurons in the brain and the median eminence. Several lines of evidence have shown that two hypothalamic neuropeptides, kisspeptin and RFamide related peptide (RFRP), are present in distinct nervous input to the GnRH neurons. While kisspeptin is a strong stimulator of GnRH secretion, RFRP is merely considered as inhibitory in most species. The mammalian RFRP precursor is processed into two biological active peptides, RFRP-1 and RFRP-3, which are characterized by a conserved C-terminal motif Arg(R)-Phe(F)-NH2. We raised a specific antiserum against the rodent RFRP-1, and used this antiserum to identify the RFRP-1 neurons in the rat brain using

immunohistochemistry. The first aim was to analyze the distribution of RFRP-1 immunoreactive (ir) neurons in the rat brain during the postnatal development (juvenile, pre-pubertal, pubertal, and adult) with special emphasis on changes at puberty onset in males and females. The second aim was to investigate if RFRP-1 neurons were affected during the estrous cycle using simultaneous detection of c-Fos in RFRP-1-ir neurons at metestrus, diestrus, proestrus and estrus. RFRP-1-ir neurons were found to be distributed along the third ventricle from the caudal part of the medial anterior hypothalamus throughout the medial tuberal hypothalamus and were localized in, as well as in between, the intermediate periventricular nucleus, dorsomedial hypothalamus, ventromedial hypothalamus and arcuate nucleus. Both the number of RFRP-1-ir neurons and the density of RFRP-1-ir/cell were unchanged during the postnatal development in male rats. However, both parameters were increased significantly in the adult (P60+) female rat. This strongly indicates a gender difference in the developmental control of RFRP-1 expression. The percentage of c-Fos positive RFRP-1-ir neurons was significantly ($P < 0.05$) higher in diestrus as compared to proestrus and estrus. This suggests that RFRP-1 neurons are indeed activated under events relevant for reproduction. All together, our data indicate that RFRP-1 plays a role in female reproduction, but whether this is manifested as inhibitory or stimulatory has still to be ruled out.

Disclosures: M.D. Andersen: None. **S.R. Jørgensen:** None. **A. Overgaard:** None. **J.D. Mikkelsen:** None.

Poster

274. **"Kndy Neurons, Kisspeptin, Rfamide"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 274.16/CCC11

Topic: E.01. Neuroendocrine Processes

Support: NOVO Nordisk Foundation PreSEED grant

Title: Kisspeptin analogues produce different effects on GnRH neurons

Authors: *J. D. MIKKELSEN[1], M. LIE[1], V. SIMONNEAUX[2], A. OVERGAARD[1]; [1]Neurobio. Res. Unit, Univ. Copenhagen - Rigshospitalet, Copenhagen, Denmark; [2]Inst. des Neurosciences Cellulaires et Intégratives, Univ. de Strasbourg, Strasbourg, France

Abstract: Kisspeptins are peptides derived from the *Kiss1* gene and are key upstream regulators of the hypothalamic-pituitary-gonadal (HPG) axis. They are all potent stimulators of gonadotropin-releasing hormone (GnRH) secretion and considered to play an essential role in regulation of pubertal maturation and reproduction. However, when kisspeptins are administered

systemically increase in LH and testosterone has been reported suggesting that kisspeptin acts directly on GnRH terminals located outside the blood-brain barrier. We here investigated the effect on the HPG axis in male rats of different (mouse (m), rat (r) and human (h) forms) kisspeptins (m/rKp10, mKp13 mKp14, mKp16, rKp16, mKp52, or hKp16) and the kisspeptin analogues (Kiss1-305 (D-Tyr-D-Pya(4)-Asn-Ser-Phe-AzaGly-Leu-Arg(Me)-Phe-NH_2), m[dY]^{1}Kp10, or h[dY]^{1}Kp10) after intraperitoneal administration. Injection of 150 nmol/kg of m/rKp10, mKp52, or hKp16 as well as the kisspeptin analogues Kiss1-305, m[dY]^{1}Kp10, or h[dY]^{1}Kp10) all produced an increase in plasma testosterone levels. Also, the other kisspeptins increased testosterone, though without reaching significance. We also examined the effect on GnRH neurons, by determining the level of c-Fos in the GnRH neurons in rats transcardially perfused 45 minutes after the injection. Interestingly, only Kiss1-305 was able to activate GnRH neurons located in the region around the organum vasculosum lamina terminalis, as well as in more distant locations in the medial preoptic area. These data suggest that kisspeptins act on GnRH terminals in the circumventricular organs i.e. the median eminence, but that more stable analogues activate GnRH neurons via another target and/or mechanisms perhaps located inside the blood-brain barrier.

Disclosures: J.D. Mikkelsen: None. **M. Lie:** None. **V. Simonneaux:** None. **A. Overgaard:** None.

Poster

275. "TIDA Neurons, Prolactin, and Lactation"

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 275.01/CCC12

Topic: E.01. Neuroendocrine Processes

Support: University of Otago Research Grant

Title: Modification of prolactin signaling in the arcuate nucleus of the mouse during lactation

Authors: *S. J. BUNN[1], S. H. YIP[2], S. E. KIRK[1], P. E. GUSTAFSON[1], D. R. GRATTAN[1]; [1]Anat., [2]Physiol., Univ. of Otago, Dunedin, New Zealand

Abstract: Prolactin is a pleotropic hormone, which acts at targets throughout the body including the mammary gland, heart, liver, muscle and brain. Its secretion from the anterior pituitary is regulated by a feedback loop in which prolactin activates the tuberoinfundibular dopaminergic (TIDA) neurons located in the arcuate nucleus of the hypothalamus. In response to prolactin these neurons synthesize and release dopamine into the hypophyseal portal system, which in turn inhibits further prolactin secretion by the pituitary lactotrophs. During lactation this negative-

feedback mechanism becomes refractory allowing circulating levels of prolactin to rise. We have demonstrated lactation is associated with a change in the phenotype of a subpopulation of TIDA neurons. By day 7 of lactation the number of enkephalin-immunoreactive cells in the arcuate nucleus of the C57/B6J mouse had risen approximately 10-fold. This response appears to be driven by prolactin in that it was suppressed by prior bromocriptine treatment (200µg/mouse every 8h for 24h) but maintained if ovine prolactin was co-administered (200µg/mouse). Over 80% of the enkephalin-positive cells seen during lactation were also immunoreactive for tyrosine hydroxylase indicating that they are TIDA neurons. Conversely these dual-labeled cells represented approximately 35% of the total TIDA neuron population. Thus prolactin stimulation of TIDA neurons may drive enkephalin synthesis during lactation but dopamine synthesis at other times. Our data suggest that a change in prolactin-activated signaling pathways may underlie this plasticity. Prolactin administration to bromocriptine-treated virgin mice generated a concentration-dependent increase in the level of both activated signal transducer and activator of transcription-3 (phospho-STAT3), and extracellular receptor regulated kinase 1/2 (phospho-ERK1/2) in TIDA neurons. The same treatment of lactating mice resulted in a phospho-STAT3 response of similar magnitude, although it required a 3-fold higher prolactin concentration, but a complete loss of phospho-ERK1/2. In conclusion these data demonstrate a lactation-associated change in the manner TIDA neurons respond to prolactin. This alteration in signal-transduction may be responsible for the switch from a dopaminergic to enkephalinergic phenotype in a subpopulation of these neurons. The physiological significance of this event is yet to be fully investigated but is likely to have implications in supporting the elevated prolactin levels essential for lactation.

Disclosures: S.J. Bunn: None. **S.H. Yip:** None. **S.E. Kirk:** None. **P.E. Gustafson:** None. **D.R. Grattan:** None.

Poster

275. "TIDA Neurons, Prolactin, and Lactation"

Location: Halls B-H

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Topic: E.01. Neuroendocrine Processes

Support: FAPESP 2011/09816-8

FAPESP 2010/52068-0

CAPES

CNPq

Title: Anatomical substrate for the neuroendocrine role of the incerto-hypothalamic area

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Abstract: The incerto-hypothalamic area (IHy) is a diencephalic region characterized by the presence of melanin-concentrating hormone (MCH)-producing neurons. Recent studies suggest that MCH-ir cells of the IHy are probably involved in the neuroendocrine control of female reproduction in specific metabolic states. In this way, our aim is to study the anatomical substrate to the neuroendocrine function of the IHy in normal female Sprague-Dawley rats. Using the retrograde neuronal tracer Fluorogold (FG) and anterograde neuronal tracer Biotinylated Dextran Amine (BDA), we observed that, in diestrus female rats, IHy is predominantly innervated by the lateral septal nucleus, median preoptic nucleus and thalamic paraventricular nucleus. IHy neurons projects mainly to the lateral septal nucleus, anteroventral periventricular nucleus, periventricular hypothalamic nucleus, medial preoptic area, medial preoptic nucleus and arcuate nucleus. Our previous studies in males have failed to show IHy projections to these neuroendocrine control areas, suggesting that IHy presents sexual dimorphic connections. Further studies are needed to unravel the neurochemical characteristics of this dimorphic innervation provided by the IHy in female rats and its role in the neuroendocrine control. Besides, there is a relative increase in the preproMCH mRNA expression in the IHy during the proestrus compared to the diestrus phase as showed by the *in situ* hybridization. The increase in the expression of the mRNA precursor is also accompanied by an increase in the number of MCH immunoreactive cells in the IHy. These data indicate that MCH is a potential candidate to mediate the neuroendocrine role exerted by the IHy.

Disclosures: D.N.M. Bueno: None. **R.J. da Silva:** None. **J.M. da Silva:** None. **J.C. Bittencourt:** None. **L.V. Sita:** None.

Poster

275. **"TIDA Neurons, Prolactin, and Lactation"**

Location: Halls B-H

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CAPES

CNPq

Title: Expression and immunoreactivity of mchr1 in mammary gland of the rat during lactation

Authors: *D. S. BATAGELLO[1,3], R. J. DA SILVA[1], H. C. DA COSTA[1], J. M. DA SILVA[1], C. F. P. LOTFI[1], L. V. SITA[1], E. M. A. F. BEVILACQUA[2], J. C. BITTENCOURT[1,3];
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Abstract: Introduction: The melanin-concentrating hormone (MCH) mRNA, as well as the MCH, are located predominantly in hypothalamic nuclei. Novel sites of MCH mRNA expression and immunoreactivity during lactation final period have been identified in other hypothalamic nuclei, such as the ventral part of medial preoptic area (MPOAv). The MCH receptor 1 (MCHR1) is an orphan G protein-coupled receptor detected in many regions of the rat brain, but there are no descriptions of MCHR1 expression in the mammary glands of non-lactating or lactating dams. Objective: this project was designed to detect MCHR1 mRNA expression and immunorreactivity in the mammary glands of lactating and non-lactating rats, correlating MCH mRNA expression in the MPOAv with the consequent MCHR1 mRNA expression in the mammary gland, thereby, increasing the knowledge regarding the control of maternal behavior (MB). Material and methods: We used in situ hybridization and immunohistochemistry to detect MCHR1 in mammary gland tissue from four groups of rats : virgins; dams of lactation day 5; dams of lactation day 12; and dams of lactation day 19. Results: We detected MCHR1 mRNA in the mammary glands of dams, in the lactation days 12 and 19, a comparable expression in the controls, in the stroma; a differential expression was found in the glandular tissue of dams of lactation day 12 compared with day 19. By immunohistochemistry, we found MCHR1-ir cells in hair follicle in skin. In glandular tissue we found cells MCHR1-ir bordering the acini, compatible with mRNA expression of MCHR1 seen in lactation day 12, but not in the day 19. Conclusion: Once that there is a differential expression of MCHR1 mRNA and MCHR1-ir cells in different parts of the mammary gland during the lactation days, this data suggests that MCHR1 is involved at the end of the lactation process.

Disclosures: D.S. Batagello: None. **R.J. da Silva:** None. **H.C. da Costa:** None. **J.M. da Silva:** None. **C.F.P. Lotfi:** None. **L.V. Sita:** None. **E.M.A.F. Bevilacqua:** None. **J.C. Bittencourt:** None.

Poster

275. **"TIDA Neurons, Prolactin, and Lactation"**

Location: Halls B-H

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Topic: E.01. Neuroendocrine Processes

Support: ERC Starting Grant (261286)

Swedish Research Council (2010-3250)

Internal funds from Karolinska Institutet

Title: Morphological characterization of tuberoinfundibular dopamine (TIDA) neurons in rodents

Authors: *A. HELLYSAZ[1], D. J. LYONS[2], C. BROBERGER[1];
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Abstract: Tuberoinfundibular dopamine (TIDA) neurons, located in the dorsomedial arcuate nucleus (DM-ARC) of the hypothalamus, control milk production and other aspects of reproduction by tonically releasing dopamine into the portal capillaries in the median eminence to inhibit prolactin secretion from the pituitary. The electrophysiological properties of rat TIDA neurons have recently been described, including a robust gap junction-dependent membrane potential oscillation that is synchronized between cells. To better understand the anatomical substrate for TIDA network oscillations we are studying the morphological features of these neurons in the rodent. During whole-cell recording of coronal male rat hypothalamic slices (P21-30), oscillator neurons in the DM-ARC were filled with neurobiotin and subsequently recovered with avidin-fluorescence histochemistry. Recorded slices were also counterstained through immunofluorescence using antibodies against tyrosine hydroxylase (TH) to ascertain the catecholaminergic nature of oscillator neurons. Three-dimensional reconstruction and the visualization were performed through fluorescence and confocal microscopy. In addition 6-12 w-old male Sprague-Dawley rats and C57BL/6 mice were perfused with a formalin-based fixative and the brain was sectioned on a cryostat. Hypothalamic sections were stained with immunofluorescence for TH to determine the rostrocaudal distribution of neurons. In sections from perfused animals, TH-immunoreactive (-ir) cell profiles could be observed at Bregma -1.7 to -3.5, *i.e.* the entire rostrocaudal extent of the arcuate nucleus with the exception of the caudal pole. Overall, the total number of TH-ir cell profiles was lower in mice compared to rats. All (n = 29) recovered oscillator neurons were TH-ir. They have an oval soma shape with the dimensions 9.8±0.6 x 16.7±0.8 µm (mean ± SEM, n = 7). No significant difference could be observed in soma size between recorded oscillator neurons and nearby TH-ir neurons (n = 18). On average, oscillator neurons have three primary dendrites (range: two-six; n = 20) which branch sparsely within the ARC. Spines are rarely, if ever, observed on the dendrites. These data reveal several distinguishing features of oscillating TIDA neurons. The sparse branching and paucity of dendrites indicate morphological characteristics that may impact on the electrical repertoire of these cells.

Disclosures: A. Hellysaz: None. **D.J. Lyons:** None. **C. Broberger:** None.

Poster

275. **"TIDA Neurons, Prolactin, and Lactation"**

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Topic: E.01. Neuroendocrine Processes

Support: European Research Council starting grant 261286

The Swedish Research council 2010-3250

Internal funds from Karolinska Institutet

Title: Oxytocin excites rat tubero-infundibular dopamine (TIDA) neurons *In vitro*

Authors: *V. BRIFFAUD, C. BROBERGER;
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Abstract: Lactation, and other functions that allow mothers to care for their offspring, is stimulated by the hormone, prolactin (PRL). Under most conditions PRL release from the pituitary is under tonic inhibition by neuroendocrine tubero-infundibular dopamine (TIDA) neurons of the hypothalamic arcuate nucleus. Successful nursing requires not only production of breast milk, stimulated by PRL, but also its ejection, promoted by the hormone, oxytocin (OT), suggesting that the release of these two hormones is coordinated. Here, we have explored the actions of OT on TIDA neuron electrophysiology. Whole-cell patch-clamp recordings were performed at room temperature from TIDA cells in acute hypothalamic slices from 21-30 day-old male rats. As previously shown, TIDA neurons exhibited a spontaneous robust ~ 0.05 Hz oscillation of the membrane potential between quiescent states and periods of depolarization crowned by action potentials. In the presence of OT (1 µM), TIDA cells depolarized and rhythmic activity switched to tonic firing (n = 6/6). This effect was mediated by a direct post-synaptic mechanism since it persisted when fast ionotropic transmission was pharmacologically blocked. In the presence of tetrodotoxin (500 nM, which abolished the rhythmic activity), OT (1 µM) induced a depolarization (+10.4 ± 2.4 mV, n = 6/8). This depolarization was abolished in the presence of the OT receptor antagonist, (d(CH2)51,Tyr(Me)2,Thr4,Orn8,des-Gly-NH29)-Vasotocin. The conductances underlying the OT effect were studied in voltage clamp recordings where OT induced a net inward current (13.16 ± 3.61 pA at a holding potential of -60 mV; n = 8/8). Preliminary findings indicate that this OT-induced current is diminished when extracellular Na^+ is substituted with Tris-HCl, and abolished in the presence of 2-Aminoethoxydiphenyl

borate (2-APB). The OT-induced current was, however, not significantly affected when extracellular Ca^{2+} was reduced to near-zero concentration. Together these results indicate that OT-induced depolarization of TIDA cells is mediated via a canonical transient receptor potential (TRPC)-like conductance. These findings suggest that OT may modulate PRL secretion by exciting TIDA neurons leading to increased dopamine release and, in turn, inhibition of PRL secretion. This work was support by grants from the European Research Council, the Swedish Research Council to C.B and internal funds from Karolinska Institutet.

Disclosures: V. Briffaud: None. **C. Broberger:** None.

Poster

275. **"TIDA Neurons, Prolactin, and Lactation"**

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Topic: E.01. Neuroendocrine Processes

Support: European Research Council starting grant 261286

Swedish Research Council 2010-3250

Internal funds from Karolinska Institutet

Title: Dopamine autoregulation of tuberoinfundibular dopamine (TIDA) neurons in the male rat: Electrophysiological studies *In vitro*

Authors: *A. S. STAGKOURAKIS, D. J. LYONS, C. BROBERGER;
Karolinska Institutet, Stockholm, Sweden

Abstract: Neuroendocrine tuberoinfundibular dopamine (TIDA) neurons in the hypothalamic arcuate nucleus regulate reproduction by providing tonic inhibition of pituitary release of the hormone, prolactin. As in all neuroendocrine axes, feedback regulation at several levels is key to maintaining appropriate serum hormone concentrations. Unlike other dopamine neurons TIDA cells have, however, been proposed to lack autoreceptors. Yet, the effects of dopamine receptor activation have not been studied on the passive and active membrane properties of the TIDA network.

Here, whole-cell recordings were performed on male rat (P21-30) hypothalamus slices maintained in oxygenated artificial cerebrospinal fluid at room temperature under differential interference contrast visualization. In the dorsomedial arcuate nucleus a neuron population exhibiting a robust oscillation (0.05 Hz) between depolarized states crowned by action potentials

and quiescent hyperpolarized states has been previously identified as TIDA cells (DJ Lyons et al., 2010, Neuron). Application of dopamine (20μM; n=5/5) significantly increased the duration of the slow depolarizing phase of the hyperpolarized state to 165.3±18.3% (average±SD) of control, the duration of the depolarized state (160.8±46.6% of control), the number of action potentials per cycle (210.5±111.1% of control) and decreased the frequency of the TIDA oscillation (71.4±9.5% of control). Application of the broad-spectrum dopamine agonist, apomorphine (20μM; n=5/5), as well as the D2-like receptor (D2R) agonist, quinpirole (20μM; n=5/5), resulted in similar changes, whereas application of the D1R-like agonist, SKF81297 (10μM, n=5/5), did not affect TIDA oscillation properties. In the presence of tetrodotoxin (500nM) application of quinpirole resulted in hyperpolarization (-3.4±1.9mV; n=7/7), suggesting the involvement of direct post-synaptic mechanisms. During application of the D2R antagonists, eticlopride (20μM; n=5/5) or haloperidol (10μM; n=5/5), TIDA behaviour changed dramatically; the 0.05Hz oscillation was replaced by a faster fluctuation at a depolarized membrane potential (ca. -45mV) and action potential discharge ceased, possibly due to depolarization block. These findings indicate that the D2R pathway is constitutively active on the TIDA network in our slice preparation, and that dopamine may regulate its own release by modulation of TIDA electrophysiological properties.

Disclosures: A.S. Stagkourakis: None. **D.J. Lyons:** None. **C. Broberger:** None.

Poster

275. "TIDA Neurons, Prolactin, and Lactation"

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Topic: E.01. Neuroendocrine Processes

Support: Fellowship from CONACyT: 45270

CONACyT: 181334

CONACyT: 156413

Title: Artificial rearing condition increases dopamine and DOPAC concentration in pituitary in neonatal rats

Authors: *C. G. TORIZ[1], B. GARCÍA IGLESIAS[1], M. MENDOZA GARRIDO[1], M. DE LA VEGA GONZÁLEZ[1], V. GARCÍA GARCÍA[2], E. SANCHEZ SANCHEZ[3], A. I. MELO SALAZAR[4], E. ESCARTÍN PÉREZ[1], B. FLORÁN GARDUÑO[1], C. SOLANO AGAMA[1], J. HERNÁNDEZ FALCÓN[5], M. GONZÁLEZ DEL PLIEGO OLIVARES[6], I. JIMÉNEZ

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Abstract: In the mature rat the tubero-infundibular dopamine (TIDA) secretion regulates pituitary prolactin (PRL) release. Likewise, TIDA system is modulated by serotonin from dorsal raphe nucleus (DRN). According to Ugrumov et al. (2010, 2012), during the first two weeks of life dopamine (DA) and serotonin (5-HT) serum levels are higher than those observed in adult rats. During this short period, blood brain barrier is open and close at 14 th day. In addition, at this period both maternal cares, such as milk products, are well known to be crucial for social and neuroendocrine development. We have observed low levels of serum PRL at 14 postnatal day (pnd) in male pups, but not at 7 or 21 pnd, in rats reared in artificial condition (AR) in comparison to males reared by their mother (MR) (Toriz et al., 43rd SfN Annual Meeting). Then, we asked if the TIDA and the hypothalamus 5-HT levels are affected in the neonatal rat under artificial reared condition. In order to answer this question we use the paradigm of AR. Male rat pups of 3 pnd were reared by their mother (MR, control) or under artificial reared condition (AR). At pnd 7, 14 or 21 we measured 5-HT and its metabolite 5-HIAA, dopamine (DA) and its metabolite DOPAC by HPLC-electrochemical detection in the hypothalamic tuberal and infundibular regions, as well as in the DRN and pituitary gland. In the DRN we found a decrease in 5-HT concentration in AR animals at 14 and 21 pnd with respect to MR animals (T test, P=0.09, P=0.02, respectively). However, hypothalamic and pituitary 5-HT concentrations did not show differences. On the other hand, DA and DOPAC levels in the pituitary were higher in AR pups of 14 pnd (T test, P=0.03, P=0.006, respectively).The present study shows that artificial rearing condition increases DA concentration in pituitary, while 5HT exhibits no-change at this level suggesting immaturity of the serotonin TIDA regulation. On this account, the low PRL serum levels observed in AR animals could be the result of the high dopamine levels observed at pituitary levels.
Ugrumov et. al. (2012). MOL CELL ENDOCRINOL. 348(1):78.
Ugrumov (2010). NEUROCHEM RES. 35(6):837.

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Poster

275. "TIDA Neurons, Prolactin, and Lactation"

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant HD072056

Title: Changes in SK3 channel and associated proteins during pregnancy and lactation in the rat supraoptic nucleus (SON)

Authors: *G. CHANDAKA[1], L. WANG[1], S. E. SENOGLES[2], W. E. ARMSTRONG[1]; [1]Dept. of Anat. and Neurobio. and Neurosci. Inst., Univ. of Tennessee, Memphis, TN; [2]Dept. of Mol. Sci., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: The physiological demands of parturition and lactation lead to the increased pulsatile release of oxytocin (OT) into the circulation from the neurohypophysial axons of OT neurons in the supraoptic (SON) and paraventricular nuclei. Calcium-dependent afterhyperpolarizations (AHPs) are enhanced in OT neurons during these periods, which would sculpt bursts underlying the pulsatile release. The plasticity of these AHPs is dependent on central OT receptor activity, mimicked by exposure to OT and estrogen, and occurs in the absence of detectable changes in voltage-gated somatic or proximal dendritic calcium currents. The calcium calmodulin (CaM) sensor of some SK3 channels plays a major role in modulating these channels via the CaM binding proteins, casein kinase 2 (CK2, which phosphorylates channel-bound CaM) and protein phosphatase 2A (PP2A, which dephosphorylates channel-bound CaM). Enhancement of AHPs could be due to an increase in the channel number (e.g., SK3 channels) or conductance, and/or due to an increase in the calcium sensitivity of the channels. We are investigating the differences in the expression pattern of SK3 channels, CK2, PP2A among virgin, late pregnant (E20-21) and lactating rats using western blots. To date, we have found that SK3 protein levels from the SON across the three groups of rats did not significantly change relative to beta-tubulin, suggesting that an increased channel density may not play a major role in the AHP enhancement. Analysis of CK2 and PP2A are in progress along with phospho-MAPK (phospho-ERK1/2), the latter of which we previously have observed upregulated in OT neurons in late pregnant rats using immunocytochemistry. Supported by NIH grant R01-HD072056 (WEA).

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Poster

275. "TIDA Neurons, Prolactin, and Lactation"

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 275.09/DDD8

Topic: E.01. Neuroendocrine Processes

Title: Fasting decreases tuberoinfundibular dopaminergic neurons and induces PRL secretion

Authors: *T. KUBOTA, M. FURUTA, A. FUKUSHIMA, C. KAKEHASHI, T. FUNABASHI, T. AKEMA;
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Abstract: Dopamine neurons located in the arcuate nucleus (ARC) and adjacent periventricular region of the mediobasal hypothalamus are called tuberoinfundibular dopaminergic (TIDA) neurons (A12). A part of their functions is to inhibit prolactin (PRL) release from the anterior pituitary as a PRL inhibitory factor. Sulpiride, a dopamine D2 receptor blocker, acts as an antidepressant and stimulates food intake, suggesting that TIDA neurons are involved in the controlling feeding behavior but its role on feeding states is not documented. We first investigated TIDA neurons during fed and fasted condition. Transgenic mice carrying green fluorescent protein under the control of the rat tyrosine hydroxylase gene (Matsushita et al, J Neurochem 2002) were used. In fed and fasted male mice at the age of 8 weeks, whole-cell voltage-clamp techniques in acute slice were applied. TIDA neurons were identified by fluorescence microscopy. The amplitude of miniature excitatory post synaptic current (mEPSC) in fasted mice was significantly decreased compared to that in fed mice, but the mean frequency of mEPSC was not different. We next investigated Fos expression by fasting in TIDA neurons. 24-fasting induced c-fos expression in the ARC, but the number of Fos-positive TIDA neurons were decreased by fasting. Low amplitude and decreased Fos expression of TIDA neurons by fasting showed that fasting lowered TIDA neurons activations. We finally measured PRL and the negative feedback of PRL since PRL acts at TIDA neurons and activates signal transducer and activator of transcription 5 (STAT5). PRL level was increased and expression of phosphorylated STAT5 was increased by fasting. All together, the present study revealed that fasting decrease excitatory synaptic inputs to TIDA neurons. As the result, PRL was high and this phosphorylated STAT5. However, pSTAT5 did not stimulate TIDA neurons. We assume that this status was a PRL resistance. Since Cytokine-Inducible SH2 Domain-Containing Protein (CIS) was not changed in the hypothalamus during feeding condition, a mechanism for PRL resistance is currently unknown, but synaptic input may be dominant role for controlling TIDA neuronal activity to release dopamine.

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Poster

275. "TIDA Neurons, Prolactin, and Lactation"

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Topic: E.01. Neuroendocrine Processes

Support: DGAPA-UNAM

CONACyT

Title: The role of suckling or non-suckling stimulation on the prolactin effects upon wide dynamic range cells of male rats

Authors: *A. GONZALEZ-HERNANDEZ[1], F. MENA[2], N. NAVARRO[2], A. CASTILLA[2], T. MORALES[2], G. ROJAS-PILONI[1], G. MARTINEZ-LORENZANA[1], M. CONDES-LARA[1]; [1]Neurobiologia del Desarrollo y Neurofisiologia, [2]Neurobiologia Celular y Mol., Inst. de Neurobiologia, UNAM, Queretaro, Mexico

Abstract: Since its discovery, considerable literature has built up describing more than 300 biological actions of prolactin. More recently it has been shown that PRL generated during peripheral inflammation could act as a mediator to reduce the pain threshold. Furthermore, in cultured trigeminal nociceptive neurons, exogenous PRL is able to sensitize TRPV1 channels and consequently a potential pro-nociceptive effect was suggested. Paradoxically it has been reported that an increase of prolactin secretion by suckling reduces pain sensitivity. We hypothesize that this difference could be attributable to the fact that PRL secreted from the adenopituitary (AP) is transformed into several molecular variants by suckling stimulation. Indeed, PRL has as a number of molecular isoforms produced by posttranslational modifications, and it has been proposed that this molecular heterogeneity is one of the mechanisms involved in the pleiotropic activity of this peptide.
In order to test the potential effect of PRL from the AP of suckled (S) or non-suckled (NS) rats on the neuronal activity of nociceptive fibers, we recorded in anaesthetized male Wistar rats the nociceptive responses evoked on the wide dynamic range (WDR) neurons. The WDR neurons are large, second-order neurons that are widely distributed in the dorsal horn spinal cord and receive input from the first-order non-nociceptive (Aβ-fibers) and nociceptive (Aδ- and C-fibers) neurons. These cells represent an important connector between primary afferent nociceptive fibers and higher nociceptive centers. Our results show that spinal administration of NS-PRL or S-PRL had no effect on the neuronal activity corresponding to activation of non-nociceptive Aβ-fibers. However, the firing of Aδ-fibers and C-fibers (both nociceptive) were: (i) increased by

NS-PRL and (ii) diminished by S-PRL. Finally, either NS-PRL or S-PRL enhanced the post-discharge activity.
These findings provide a basis for addressing the physiological relevance of the prolactin variants induced by suckling and might help to explain the differences observed in several experiments related to nociception.

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Poster

275. **"TIDA Neurons, Prolactin, and Lactation"**

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Topic: E.01. Neuroendocrine Processes

Support: E26/103.193/2011

CNPq, FAPERJ, CAPES

Title: Behavioral effects in the offspring of maternal protein malnutrition during lactation

Authors: *J. D. SILVA, M. C. F. FRAGA, F. N. NUNES, E. G. DE MOURA, P. C. LISBOA, C. C. FILGUEIRAS, Y. D. VILLAÇA, A. C. MANHÃES;
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Abstract: Neonatal undernutrition in rats results in short- and long-term metabolic and endocrine alterations in the offspring. It is known that some of these alterations, such as adrenal catecholamines content and hypercorticosteronemia, are associated with behavioral alterations in adult animals. However, it is not known whether the alterations are present since the original insult or if these arise at some specific point after it. In this sense, in the present work, we analyzed, in Wistar rats, the effects of protein restriction programing during lactation on behavioral parameters associated with anxiety, locomotor activity and memory/learning at different time points during development and maturation. Progenitors were separated into 2 groups after delivering: PR (protein restriction) group - which received hipoproteic chow (8% protein) from birth to weaning [postnatal day (P) 21], and CONT (control) group - which received normoprotein chow during this period. The following behavioral tests were used to analyze offspring behavior (21 animals per group/age) at P21, P45, P90 and P180: 1) elevated plus maze (EPM) - to assess anxiety related behaviors; 2) open field (OF) - to assess locomotor

activity; 3) radial arm water maze (RAWM) - to assess memory and learning. After the behavioral tests, animals were decapitated and blood and the adrenal glands were collected for the analyses of catecholamine content and serum corticosterone level. In the EPM, PR animals had a significant increase in the values of the anxiety-associated variables when compared to the control group at P21. In the OF, a significant reduction in the number of traversed rectangles was observed in the PR group at P90. In the RAWM, we did not identify significant differences between groups during the first four days of testing in all ages. On the fifth day (probe trial), the PR group had a significant reduction in the latency to find the scape platform at P21 and P180. As for the endocrine data, the PR group had a significant reduction in serum corticosterone level at P90. Catecholamine content was increased in the RP group at P21 and P180. Our data indicate that animals in the PR group display: 1) a reduction in anxiety-like behaviors at weaning; 2) a reduction in locomotor activity as young adults; 3) an improvement in memory/learning performance both at weaning and as mature adults. Moreover, our results indicate that there is an association between behavioral and endocrine alterations. We can conclude that protein restriction during lactation in rats affects behavior at different time points during the animal's life and that the temporal profile of the effects varies as a function of the behavior that is being assessed.

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Poster

275. **"TIDA Neurons, Prolactin, and Lactation"**

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Topic: E.01. Neuroendocrine Processes

Support: UC CRCC-43674

NIDA DA024689

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Title: Each individual isoform of the dopamine D2 receptor protects from lactotroph hyperplasia

Authors: D. RADL, H. LEE, *E. BORRELLI;
Univ. California, IRVINE, CA

Abstract: Dopamine acting through D2 receptors (D2R) controls lactotroph proliferation and prolactin (PRL) levels. Ablation of this receptor in mice results into lactotroph hyperplasia and prolactinomas in aged females. Alternative splicing of the Drd2 gene generates two independent isoforms, a long (D2L) and a short (D2S), which are present in all D2R expressing cells. Here, we addressed the role of D2L and D2S on lactotrophs physiology through the generation and analysis of D2S-null mice and their comparison to D2L-null animals.
These mice represent a valuable tool to investigate dopamine-dependent isoform-specific signaling in the pituitary gland. We sought to assess the existence of a more prominent role of D2L or D2S in controlling PRL expression and lactotroph hyperplasia. Importantly, we found that D2L and D2S are specifically linked to independent transduction pathways in the pituitary. D2L-mediated signaling inhibits the AKT kinase activity while D2S is instead required for the activation of the ERK 1/2 pathway. Under normal conditions, presence of only one of the two D2R isoforms in vivo prevents hyperprolactinemia, formation of lactotroph's hyperplasia and tumorigenesis that is observed when both isoforms are deleted as in D2R-/- mice. However, the protective function of the single D2R isoforms is overridden when single isoform knockout mice are challenged by chronic estrogen treatments as they show increased prolactin production and lactotroph hyperplasia. Our study indicates that signaling from each of the D2R isoforms is sufficient to maintain lactotroph homeostasis in physiological conditions, however signaling from both is necessary in conditions simulating pathological states.

Disclosures: D. Radl: None. **E. Borrelli:** None. **H. Lee:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 276.01/DDD12

Topic: E.02. Neuroimmunology

Support: MINECO Grant SAF 2010-17501

REEM Grant RD0700100060

Title: Alterations in the endocannabinoid machinery in alternative microglia *In vitro*

Authors: M. MECHA[1], A. FELIÚ[1], F. CARRILLO-SALINAS[1], *C. GUAZA[2];
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Abstract: The regulation of inflammation in the central nervous system (CNS) is necessary to prevent the irreversible cellular damage that occurs in neurodegenerative diseases like Multiple

Sclerosis. Under pathological conditions, much is known about how microglial cells play a pivotal role becoming rapidly activated, changing both phenotype and morphology, and displaying several functions like antigen presentation, phagocytosis and cytokine production. On the other hand, little is known about how microglial cells serve a protective role in the CNS, acquiring an alternative state (also referred to as M2-type microglia) that is associated with tissue repair, anti-inflammatory cytokine production and extracellular matrix reconstruction. Compared to classically activated microglia, NOS2, IL-12, IL-1b, IL-6 and TNFa induction are suppressed in alternative activated microglia when stimulated with IL-4 and IL-13, whereas repair genes like Arginase1 and Ym-1 are activated. However, the participation of the endocannabinoid machinery in the acquisition of the M2-type microglia phenotype has not been addressed yet.

In this study, we have investigated the possible alterations of the endocannabinoid system in the alternative activated primary microglia *in vitro*, stimulated with the anti-inflammatory cytokines IL4 and IL13. M2-type microglia cells showed an increase in the Arginase-1 and IGF-1 levels, as it has been previously described in M2-type macrophages. Using RT-PCR, we analyzed the mRNA levels of the endocannabinoid classical immune receptor (CB2), and of the enzymes of synthesis (NAPE and DAGL) and degradation (FAAH and MAGL) of AEA and 2-AG, respectively. M2-type microglia express a higher expression of CB2 and of the synthesis enzyme of AEA (NAPE); moreover, this alternative activated cells express lesser expression of both degradation enzymes (FAAH and MAGL), suggesting a change in the endocannabinoid system towards an increase in the production and signaling of classical cannabinoids like AEA and 2-AG .

Our results highlight the importance of the endocannabinoid system in the alternative state of microglia, and open a new field of study in the repair functions of immune cells in the CNS.

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Disclosures: M. Mecha: None. **A. Feliú:** None. **F. Carrillo-Salinas:** None. **C. Guaza:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Topic: E.02. Neuroimmunology

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DOD AL120029

Title: Identification of unique molecular and functional signature in microglia

Authors: *O. BUTOVSKY[1,2], M. JEDRYCHOWSKI[3], C. MOORE[4], A. LANSER[1], T. KOEGLSPERGER[5], B. DAKE[6], R. CIALIC[1,2], P. WU[1], C. DOYKAN[1], S. GYGI[2], J. ANTEL[4], H. WEINER[1];
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Abstract: Microglia are myeloid cells of the central nervous system (CNS) that participate both in normal CNS function and disease. A barrier to the study of microglia is the lack of markers that distinguish them from infiltrating monocytes during disease. The distinction between resident microglia and recruited mononuclear phagocytes is crucial, because it provides an approach to understand the biology of microglia and their relevance to disease. We have identified a microRNA/gene/protein signature that is uniquely expressed in resident adult microglia in mice and humans. This molecular microglial signature was not observed in classically studied microglial cell lines or embryonic preparations of microglia, nor in peripheral immune cells or recruited mononuclear cells in animal models of MS, AD or ALS. We identified 239 genes and 8 microRNAs that are uniquely or highly expressed in microglia. We found unique microglial surface markers related to the TAM system and targeting of these receptors modulated the microglial phenotype. Furthermore, we found a crucial role for TGF-β in microglial biology including the absence of microglia in CNS-TGF-β deficient mice. We also identified unique patterns of microglial dysfunction associated with CNS disease in EAE, SOD1 and APP/PS1 mouse models. Most of the identified unique microglial genes were downregulated during acute or chronic phases in EAE mice or during disease progression in SOD1 and APP/PS1 mice. Our identification of a unique microglial signature provides a new understanding of microglial biology and the possibility of targeting microglia for the treatment of CNS disease.

Disclosures: O. Butovsky: None. **M. Jedrychowski:** None. **C. Moore:** None. **A. Lanser:** None. **T. Koeglsperger:** None. **B. Dake:** None. **R. Cialic:** None. **P. Wu:** None. **C. Doykan:** None. **S. Gygi:** None. **J. Antel:** None. **H. Weiner:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

Location: Halls B-H

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Program#/Poster#: 276.03/EEE2

Topic: E.02. Neuroimmunology

Support: NIH Grant MH 093854

Title: Morphological differences in microglia in the mouse brain through development may contribute to vulnerability to stressors during puberty

Authors: *M. K. HOLDER, J. D. BLAUSTEIN;
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Abstract: The peak age of onset for diseases of mental health is during puberty, with extremely stressful or traumatic experiences during this period contributing to a increased risk for affective disorders. In female mice, the experience of shipping from a supplier or a single injection of the bacterial endotoxin, lipopolysaccharide (LPS) during pubertal development alters the behavioral response to estradiol in adulthood as demonstrated by perturbations of estradiol's anxiolytic and anti-depressive properties. Microglia, the primary type of immunocompetent cell, contribute to brain development and respond to stressors with dramatic and long-lasting morphological and functional changes. Recently, we have begun to investigate the role that microglial cells may play in mediating the alteration in hormone response that results from pubertal stressors. We first examined the basal morphology of microglia during (6 wks old) and after (10 wks old) the pubertal period of female mice using immunohistochemistry for ionized calcium binding adaptor molecule 1 (Iba1), which is constitutively expressed in microglia in areas implicated in anxiety and depression. Interestingly, age of animal was without effect on the total number of microglia in each brain area. However, we observed more hyper-ramified, or activated, microglia in the hippocampus and amygdala at 6 wks than at 10 wks. These data suggest that the developmental status of the microglia may underlie the alteration in hormone-responsive affective behaviors. We are currently examining the microglial morphology following the experience of either an immune (LPS) or a shipping stressor in pubertal female mice. Supported by MH 093854 from the National Institutes of Health.

Disclosures: M.K. Holder: None. **J.D. Blaustein:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Program#/Poster#: 276.04/EEE3

Topic: E.02. Neuroimmunology

Support: BrightFocus Foundation

Title: The role of microglial-specific MyD88-dependent signaling after LPS stimulation

Authors: *C. M. MILLER[1], K. BHASKAR[2], C. PARKHURST[3], D. LITTMAN[3], W.-B. GAN[3], R. RANSOHOFF[1], B. LAMB[1];
[1]Neurosciences, The Cleveland Clin. Lerner Res. Inst., Cleveland, OH; [2]Univ. of New Mexico, Albuquerque, NM; [3]New York Univ., New York, NY

Abstract: As the resident immune cells of the CNS, activated microglia strongly influence the state of neuroinflammation by the cytokines and chemokines they produce, thus polarizing a response toward either pro- or anti-inflammatory depending on the stimulus. Peripheral stimulation with lipopolysaccharide (LPS) signals through *t*oll-*l*ike-*r*eceptor *4* (TLR4) on microglia and promotes their activation and a pro-inflammatory response. Interestingly, stimulation with LPS can also enhance intraneuronal *m*icrotubule-*a*ssociated *p*rotein *t*au (MAPT) pathologies, including increases in phosphorylation and aggregation of MAPT, both characteristic of tauopathies like Alzheimer's disease. TLR4 is an innate immune receptor, whose downstream signaling converges through the universal adaptor protein *my*eloid *d*ifferentiation primary response gene *88* (MyD88). Notably, MyD88-dependent signaling activates transcription factors responsible for the regulation of many downstream pro-inflammatory cytokines and chemokines. However, the relationship between MyD88-dependent signaling within microglia and development of neuronal MAPT pathologies has not been explicitly tested, due to the fact that most studies have utilized MyD88 knock-out mice that remove MyD88 from all cell types within the brain and periphery.
In order to assess the role of MyD88-dependent microglial activation on the development and progression of MAPT pathology, we generated mice in which MyD88 can be conditionally removed specifically from microglia ($Cx3cr1^{CreER/+}$; $MyD88^{flox/flox}$) after tamoxifen administration. We hypothesize that abrogation of the microglial MyD88 signaling will reduce pro-inflammatory cytokine/chemokine production and attenuate MAPT pathologies after LPS injections. To test this hypothesis, $Cx3cr1^{CreER/+}$; $MyD88^{flox/flox}$ mice were administered tamoxifen at six weeks of age and two weeks later treated with LPS. Twenty-four hours after LPS stimulation, microglial MyD88-deficient mice were examined for alteration in microglial activation and neuronal MAPT pathologies. This study provides proof-of-concept for using $Cx3cr1^{CreER}$ mice as a tool to abrogate microglial signaling molecules (including MyD88) in the development and progression of neurological diseases.

Disclosures: C.M. Miller: None. **K. Bhaskar:** None. **B. Lamb:** None. **W. Gan:** None. **R. Ransohoff:** None. **C. Parkhurst:** None. **D. Littman:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Program#/Poster#: 276.05/EEE4

Topic: E.02. Neuroimmunology

Title: Lectin-based passive and active dendritic cell immunotherapies against experimental autoimmune encephalomyelitis/multiple sclerosis, and Glioblastoma

Authors: *D. SAGAR[1], C. FOSS[2], Z. K. KHAN[3], J. SHIRAZI[3], M. G. POMPER[2], P. JAIN[3]; [1]Neurobio. & Anat., Drexel Univ. Colleged of Med., Doylestown, PA; [2]Johns Hopkins Univ., Baltimore, MD; [3]Drexel Univ. Col. of Med., Doylestown, PA

Abstract: The potential of dendritic cells (DCs) as sentinels for immunotherapy is emerging against various diseases such as HIV, multiple sclerosis (MS) and various cancers. DC immunotherapy remains a promising therapeutic approach and warrants further exploration to enhance its immunogenicity. Here, we seek to utilize a specific aspect of DC biology involving the expression of surface lectin receptors that play variable roles including cell-cell adhesion and innate pathogen recognition. While studying molecular mechanisms of how circulating DCs gain access to the CNS, we found that lectins play crucial role in transmigration of DCs across the blood-brain barrier (BBB). The near-infrared imaging (NIRF) of DCs in experimental autoimmune encephalomyelitis (EAE, the model for MS), indicated that DCs' accumulation into areas of CNS correlate with the severity of inflammation. Further studies confirmed the involvement of the chemokine CCL2 in this process involving a cross talk between actin cytoskeleton and lectins. Profiling of different DC subsets- myeloid (mDC), plasmacytoid (pDC) and monocyte-derived (MDDC) revealed a unique enrichment of lectin receptors on each DC subset. Subsequently, in the presence of CCL2, each DC subset was found to utilize a different lectin type in order to adhere and transmigrate across the BBB. While lectins can be specifically blocked on DCs to prevent transmigration, they can also be harnessed to enhance cytotoxic response against tumors. Glioblastoma multiforme (GBM) is one of the most common and malignant types of brain tumor with mean survival time of 1 year or 2-3 years in case of anaplastic astrocytoma. Therefore, we used a GBM as model to test a glycotargeting approach for the uptake of glioblastoma tumor antigens via specific lectin recepors on DCs. Further, anti-HIF1α (hypoxia inducible factor 1α) shRNA was transduced into DCs to revert the effect of hypoxia. We first confirmed that hypoxic environment affects the expression of antigen-presentation, -uptake and migratory receptors on DCs that hampered their antigen presentation capability. Futher, anti-HIF1α transduced DCs were shown to efficiently migrate in vivo using NIRF. Our dual approach of lectin-targeting and hypoxia-reversal showed potentiation of DC migration and functionality under hypoxic conditions and has further potential to be tested in vivo. In conclusion, both aspects of our study will further substantiate the promise of current

DC-based immunotherapies to battle diseases that overpower the body's immune capabilities, and generate lectin-targeted therapies that can be directed to inflammatory lesions or tumors.

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Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Topic: E.02. Neuroimmunology

Support: Univ of Missouri Res Board Grant

Title: Rapid stimulation of IFN-regulated genes and JAK/STAT signaling in the brain following peripheral administration of IFN-γ

Authors: *J. WANG, Y. DAI;
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Abstract: Interferon-gamma (IFN-γ) is a pleiotropic cytokine and implicated in inflammatory and degenerative CNS disorders. Nevertheless, recent studies have identified a direct crosstalk of IFN-γ with morphogen sonic hedgehog (Shh) in neuronal precursor cells, indicating a potential importance of IFN-γ in neurodevelopment and/or neurogenesis. As a key antiviral cytokine and immune regulator, IFN-γ is produced mainly by T lymphocytes and NK (natural killer) cells in the periphery. It remains unresolved whether systemic IFN-γ acts on the brain directly or not. Here, we took an approach to analyze the expression of IFN-γ target genes in the CNS as a measurement of direct action of IFN-γ in the brain following peripheral administration. We found that systemic administration of IFN-γ by intraperitoneal injection induced a rapid and profound stimulation of IFN-stimulated genes (ISGs) both in the CNS and peripheral tissues in mice. Dose response studies showed a significant upregulation of ISGs, including CTIIA (MHC class II trans-activator gene), TGTP (T-lymphocyte GTPase) and OAS (2', 5'-oligoadenylated synthase) in brain by IFN-γ at doses as low as 0.1 μg. Doses of IFN-γ higher than 1.0 μg did not further enhance its stimulatory activity on ISG expression in both the brain and liver. Time course studies revealed that expression of ISGs in the brain peaked at around 2 hours, was attenuated at 8 hours, and returned to baseline after 24 hours following treatment. Immunoblot detected an increase in expression and phosphorylation of both STAT1 and STAT3 in the brain regions following peripheral IFN-γ treatment. In conclusion, our studies indicate a direct access of peripherally administered IFN-γ to the brain and activating IFN-γ-initiated JAK/STAT

signaling in the CNS. Together, direct action of circulatory IFN-γ on the brain underscores the significance of systemic IFN-γ in brain development and function.

Disclosures: J. Wang: None. **Y. Dai:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 276.07/EEE6

Topic: E.02. Neuroimmunology

Title: The effects of calorie restriction on fever and neuroimmune communication pathways in mice

Authors: *S. KENT, M. RADLER, G. SMITH, M. W. HALE;
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Abstract: Calorie restriction (CR) has been shown to increase longevity and elicit many health promoting benefits, including delaying the development of diabetes, improving learning and memory, and reducing anxiety. In addition, CR has been shown to attenuate fever and sickness behaviour in response to systemic immune challenges. However, it is unclear how the neuroimmune pathways that relay immune signals between the brain and the periphery are affected by CR. The current study examined the expression of c-Fos, the protein product of the immediate early gene (IEG) *c-fos*, in brain regions known to be involved in neuroimmune communication pathways. Twenty-two male C57BL-6J mice were either fed ad libitum or were subjected to a CR dietary regimen for four weeks. Following the CR period, mice were injected with saline or 50 μg/kg of lipopolysacharide from *Escherichia coli* (serotype 0111:B4). Immunohistochemistry was conducted and cells that expressed c-Fos were counted in five regions of the medulla and pons, including the lateral parabrachial nucleus, locus coeruleus, nucleus of the solitary tract and the area postrema. Lipopolysaccharide increased c-Fos expression; however, CR did not alter c-Fos expression
in the regions examined. This indicates that CR attenuation of fever and sickness behavior does not occur peripherally, but is instead moderated by central mechanisms. Neuroinflammation was also examined as a possible mechanism and data will be presented at the meeting.

Disclosures: S. Kent: None. **M. Radler:** None. **G. Smith:** None. **M.W. Hale:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

Location: Halls B-H

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Program#/Poster#: 276.08/EEE7

Topic: E.02. Neuroimmunology

Support: Alberta Innovates health Science

Title: Harnessing the benefits of polarized macrophages for CNS functions

Authors: *M. K. MISHRA, M. KEOUGH, V. W. YONG;
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Abstract: Monocytoid cells (circulating monocytes, tissue infiltrated macrophages and CNS-resident microglia) are very plastic and heterogeneous cells of innate immunity that have important roles in homeostasis and defense. While an overactive and uncontrolled inflammatory response directed towards the CNS is detrimental, it is increasingly recognized that some components of the immune response may be beneficial. Recent lines of evidence suggest that macrophage activation state can switch from a proinflammatory (M1) to an anti-inflammatory (M2) state, depending on the microenvironment. As with peripheral macrophages, microglia respond to the prevailing cytokine milieu and display markers of M1 and M2 cells. Macrophages are involved in various functions including extracellular matrix (ECM) remodeling; these ECM has inhibitory components such as chondroitin sulfate proteoglycans (CSPGs) that inhibit neuronal adhesion and neurite outgrowth. We have characterized macrophages and microglia in culture into M1 and M2 subsets by qPCR, western blot, ELISA and by nitric oxide and arginase assays. We have also examined the proteolytic properties of M1 and M2 macrophages particularly their capacity to overcome the inhibitory effects of CSPGs. We have found that M1 and M2 polarized macrophages produce different spectrum of matrix metalloproteinases, TIMPs and cytokines. The conditioned media from M1 and M2 macrophages have differential effect on neuronal survival, with M1 being toxic. Moreover, these conditioned media process CSPGs differentially, and M2 conditioned media overcome the inhibition by CSPGs on neuritic outgrowth. We have also found that vitamin D and minocycline at low concentrations reduce the proinflammatory M1 phenotype of BMDMs, and favor cells towards the M2 subset. Overall, our data suggest that the polarization of macrophages and microglia is a tool to reduce detrimental neuroinflammation and to harness the beneficial aspects of neuroinflammation to favor recovery in various CNS diseases.

Disclosures: M.K. Mishra: None. **M. Keough:** None. **V.W. Yong:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Topic: E.02. Neuroimmunology

Support: CNPq

CAPES

FAPESP

Title: Age-related changes induced by Lipopolysaccharide on α2,3-Na,K-ATPase activity, cyclic GMP levels and oxidative status in rat hippocampus

Authors: *A. R. VASCONCELOS[1], L. M. YSHII[1], A. E. BÖHMER[1], L. S. LIMA[1], R. ALVES[1], D. Z. ANDREOTTI[1], T. MARCOURAKIS[2], C. SCAVONE[1], E. M. KAWAMOTO[1,3]; [1]Dept. of Pharmacol. - Inst. of Biomed. Sci., [2]Dept. of Clin. and Toxicological Analysis - Fac. of Pharmaceut. Sci., Univ. of Sao Paulo, Sao Paulo, Brazil; [3]Natl. Inst. on Aging Intramural Res. Program, Baltimore, MD

Abstract: Chronic neuroinflammation is a common characteristic of neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, that may contribute to loss of function and cell death. Sodium, potassium pump (Na,K-ATPase) plays an important role to maintain cell ionic equilibrium. Disruption of Na,K-ATPase activity and cyclic GMP signaling could lead to oxidative stress process which could be detrimental to the cells. In this project we compared the effects of an inflammatory stimulus induced by Lipopolysaccharide (LPS, 1 mg/kg) administration on the activity of isoforms α_1 and $\alpha_{2,3}$-Na,K-ATPase, cyclic GMP and TBARS levels in hippocampus of young (4-month-old), adult (12-month-old) and aged (24-month-old) male Wistar rats. The results showed that Aging induces a progressive decrease in hippocampal total-Na,K-ATPase activity at 12 and 24 months when compared with the values detected at 4 months, which is due to a reduction in $\alpha_{2,3}$-Na,K-ATPase activity, since α_1-Na,K-ATPase and Mg-ATPase activities are not changed. Also, LPS caused a decrease of the total-Na,K-ATPase activity 2 h after intravenous injection at 4 and 12 months. This effect was also specific to $\alpha_{2,3}$-Na,K-ATPase. We also showed that cyclic GMP levels decrease at 12 and 24 months when compared to 4-month-old animals. TBARS determinations showed that aging is linked to progressive increase in products of lipid peroxidation at 12 and 24 months when compared to 4-month-old rats. Moreover, LPS treatment decreased cyclic GMP levels at 4 and 12 month-old animals but not at 24 months when compared with respectively aged control groups. On the other

hand, TBARS levels were increased after LPS at 4, 12 and 24 month-old animals when compared to respectively aged control groups. Taken together, these findings suggest that aging is associated with a progressive decrease of protective signaling related to an increased risk for deficits on brain function and neurodegenerative disorders which are aggravated by inflammation.

Disclosures: A.R. Vasconcelos: None. **L.M. Yshii:** None. **A.E. Böhmer:** None. **L.S. Lima:** None. **R. Alves:** None. **D.Z. Andreotti:** None. **T. Marcourakis:** None. **C. Scavone:** None. **E.M. Kawamoto:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Support: Korean Government, Ministry of Education, Science and Technology, National Research Foundation of Korea grant NRF-2010-355-E00048

Korean Government, Ministry of Education, Science and Technology, National Research Foundation of Korea grant NRF-860-2012-0090

Title: IL-2 activated natural killer cells interact with but do not lyse adult DRG neuron targets

Authors: *A. J. DAVIES[1], J. LEE[1], J. LEE[2], J. CHOI[1], M. KIM[2], K. LEE[2], S. OH[1]; [1]Natl. Res. Lab. for Pain, Dept. of Physiol., Sch. of Dentistry, Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Global Res. Lab, Col. of Med., Korea Univ., Seoul, Korea, Republic of

Abstract: Natural Killer (NK) cells are cytotoxic lymphocytes typically involved in cell lysis of tumour or virus-infected target cells. It has previously been shown that NK cells are cytotoxic against embryonic dorsal root ganglia (DRG) neurons and can restrict neurite outgrowth central neurons *in vitro*, however very little is known about the interactions between NK cells and the nervous system. We observed NK cell-like immunoreactivity in sections of adult mouse DRG in close-proximity to neurons and glia suggesting possible functional interactions. We first confirmed the ability of IL-2 activated NK cells to lyse embryonic mouse DRG neurons using a Cr^{51}-release assay. NK cell activity is co-ordinated by a fine balance of stimulatory and inhibitory signals. We go on to show that cytoxicity against embryonic DRG neurons is dependent on the activating NK cell receptors NKG2D and 2B4 using antibody blocking and receptor knock-out strategies. In contrast, DRG neurons cultured from adult mice were resistant

to cytotoxicity suggesting possible inhibitory receptor function. Instead we show that IL-2 activated but not resting NK cells physically interact with adult DRG neurons in co-culture by immunocytochemistry and live confocal microscopy. We also tested the ability of NK cells to lyse primary-cultured microglia. IL-2 activated NK cells showed significant cytotoxicity against resting microglia but unlike embryonic DRG lysis was not dependent on 2B4 receptor function. Conversely, prior activation of microglia with lipopolysaccharide was protective against NK cell-mediated lysis. Our results suggest that NK cell activation within the nervous system may lead to differential effects on neurons and glia respectively.

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Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Program#/Poster#: 276.11/EEE10

Topic: E.02. Neuroimmunology

Support: Emerald Foundation, Inc.

Title: Recruitment of interferon-producing killer dendritic cells to the viral-induced encephalitic brain is IL-15 dependent

Authors: *A. TROCHTENBERG, P. M. D'AGOSTINO, K. BULLOCH;
The Rockefeller Univ., New York, NY

Abstract: Interferon-producing killer dendritic cells (IKDC), defined by their $NK1.1^+$ $CD49b^+$ $B220^+$ IL-$2R\beta^+$ $CD11c^{int}$ phenotype, have been recognized in the periphery as a novel tumoricidal cell capable of both cytotoxicity and antigen presentation. However, these cells have remained uncharacterized in the infected brain. Using intranasally administered Vesicular Stomatitis Virus (VSV) as a model for viral-induced encephalitis, we identified a population of cells bearing the IKDC phenotype in the olfactory bulb (OB) 4 days post-infection (dpi). In initial studies performed on *CD11c-eyfp* mice, a transgenic C57BL/6 mouse in which enhanced yellow fluorescent protein (YFP) expression is linked to the CD11c promotor, we identified putative IKDC based on YFP expression. We observed a rare IKDC subset in the OB that was both $TRAIL^+$ and IFN-γ producing 4 dpi, substantiating our hypothesis that the identified population represented putative IKDC. In our current study, we focus on further characterizing the lineage and function of IKDC in the encephalitic brain. In order to assess the origin of these putative

IKDC, we infected IL-15$^{-/-}$ (a C57BL/6 transgenic mouse lacking cells of the NK lineage) and C57BL/6 wild-type (WT) mice using our VSV protocol. Recruitment of putative IKDC cells by VSV-infected WT and IL-15$^{-/-}$ mice was analyzed by flow cytometry, which required use of an anti-CD11c antibody (clone N418) to evaluate the CD11c^{+} population in the absence of the YFP selection marker. Our data clearly demonstrate recruitment of a large population of putative IKDC by 4 dpi in WT mice, whereas cells bearing the IKDC phenotype are absent from the olfactory bulb of IL-15$^{-/-}$ mice. This finding strongly implies that IKDC are derived from a NK precursor, reminiscent of a recently reported peripheral B220^{+} NK population (Guimont-Desrochers, *Blood*, 2012). Interestingly, we also observe that the putative IKDC population on 4 dpi is significantly larger in WT mice than that reported in *CD11c-eyfp* transgenic mice. Further flow cytometry analysis of VSV-infected *CD11c-eyfp* mice, using an anti-CD11c antibody in conjunction with the YFP reporter, demonstrates a large population of putative IKDC that are CD11c^{+} but YFP^{neg}, suggesting that this population of interest: 1) has down-regulated the CD11c promoter while retaining surface expression of CD11c protein and 2) composes a larger fraction of cells recruited to the OB than previously recognized. Identification of the expanded population of CD11c^{+} NK1.1^{+} CD49b^{+} B220^{+} IL-2Rβ^{+} cells will facilitate our further functional analysis of their cytotoxic and antigen presenting potential in VSV-induced encephalitis.

Disclosures: A. Trochtenberg: None. **P.M. D'Agostino:** None. **K. Bulloch:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Topic: E.02. Neuroimmunology

Support: NIH R01 AG-033028-04

Title: Spinal cord injury in aged mice resulted in increased lesion length and limited functional recovery associated with impaired interleukin-(IL)4 receptor-alpha mediated regulation of microglia

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Abstract: Older individuals have a poorer prognosis, worse functional recovery, and higher mortality rate after spinal cord injury (SCI). Limited studies, however, have investigated the mechanism by which SCI is worse with age. Our previous work indicates that activated microglia from aged mice are less sensitive to anti-inflammatory and reparative (M2a) signals.

Therefore, we hypothesize that impaired M2a-mediated regulation of active microglia in the aged plays a pivotal role in SCI severity. To begin to test this hypothesis, adult (3-4 mo) and aged (18-19 mo) mice were subjected to mid-thoracic (T_9) SCI. Here, we show that functional recovery after SCI was limited in aged mice compared to adults with lower Basso Mouse Scale (BMS) scores and failure to regain plantar stepping. Moreover, lesion length was 38% longer in aged mice compared to adults. To assess the reparative potential of resident and infiltrating microglia/macrophages after injury, protein expression of the M2a-driven enzyme, arginase, was determined. Coinciding with worsened functional recovery, the number of arginase$^+$ microglia/macrophages was reduced in the injured spinal cord of aged mice. Because arginase is driven by interleukin-4 (IL-4), expression of its corresponding receptor, IL-4 receptor-alpha (IL-4Rα), was determined on microglia and infiltrating monocytes after injury. Microglia from aged mice failed to increase IL-4Rα protein expression after SCI associated with a reduction in cytokines, chemokines, and trafficking of IL-4Rα$^+$ monocytes. To better understand IL-4Rα-mediated regulation of inflammatory activated microglia, a series of intracerebroventricular (i.c.v.) IL-4 infusion studies were completed. Mice were injected peripherally with lipopolysaccharide (LPS) to induce an acute inflammatory response and drive IL-4Rα expression on microglia, followed by an i.c.v. injection of IL-4. These studies show that functional IL-4/IL-4Rα interactions were critical in re-directing inflammatory activated microglia towards an M2a phenotype. Indeed, only LPS-activated microglia with upregulated IL-4Rα expression had increased arginase expression after i.c.v. IL-4. Furthermore, enhanced IL-4Rα expression on active microglia was required for IL-4-dependent neurite growth *ex vivo*. Thus, inflammatory-induced IL-4Rα expression is necessary for IL-4-driven M2a responses by microglia. Collectively, these studies indicate that impaired IL-4Rα upregulation on active microglia of aged mice limits IL-4-mediated M2a redirection contributing to increased tissue damage and worse functional outcome after SCI.

Disclosures: A.M. Fenn: None. **J.C. Hall:** None. **J.C. Gensel:** None. **P.G. Popovich:** None. **J.P. Godbout:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 276.13/EEE12

Topic: E.02. Neuroimmunology

Title: Interleukin-1beta excites rat histaminergic neurons

Authors: *H. L. HAAS, Y. YANOVSKY, O. A. SERGEEVA;
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Abstract: Members of the inflammatory cytokine interleukin-1 (IL1) family, interleukin-1beta (IL1β) and IL1 receptor antagonist (IL1RA) affect body temperature in response to systemic inflammation through the hypothalamus. Neuronal histamine prevents amplification of IL1β signalling in the hypothalamus and suppresses fever (Tringali et al., 1997; Kang et al., 1995). It was reported previously that IL1β enhances histamine turnover, histidine decarboxylase (HDC, histamine-producing enzyme) and histamine release (Niimi et al., 1994,1997). We recorded now from identified histaminergic neurons in the tuberomamillary nucleus of the hypothalamus (TMN) in cell-attached mode in rat (P22-26) brain slices during IL1β (1.7nM) bath perfusion and report an increased firing, to 200±48 % (n=5) of control. Single-cell RT-PCR revealed expression of the IL1β receptor (IL1R) by 30% of the TMN neurons. In 60% of IL1R-negative TMN neurons mRNA encoding for the IL1β peptide was detected. IL1RA transcripts were not detected in individual neurons, but were found in the whole TMN region used as positive control. Bath application of IL1RA prevented the increase of neuronal firing by IL1β (n=4). Moreover, GABA (10-30µM, n=4) or ammonium chloride (5mM, n=5) given prior to IL1β significantly suppressed excitation, most likely by inhibiting the amplification of IL1β signalling by glia or neurons. Mechanisms of glia-TMN neuron interaction may involve release of glutamate (Huang et al., 2010) and activation of metabotropic glutamate receptors of type I as we have shown previously for the response to protons (Yanovsky et al, 2012). Thus direct activation of histaminergic neurons by IL1β and amplification of interleukin signalling in the TMN region provide a cellular basis for feed-back control of the inflammatory fever response.
Huang et al (2010) Eur J Pharmacol 629:125-131. Kang et al (1995) Am J Physiol 269:R1308-R1313. Niimi et al (1994) Neurosci Lett 181:87-90. Niimi et al (1997) J Neurochem 69:851-858; Tringali et al (1997) Pharmacol Res 36(4):269-273. Yanovsky et al. doi: 10.3389/fnsys.2012.00023.

Disclosures: H.L. Haas: None. **O.A. Sergeeva:** None. **Y. Yanovsky:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 276.14/EEE13

Topic: E.02. Neuroimmunology

Support: HL96571

NS34179

NS37853

Title: Spatial, temporal and phenotypic differences in GFP^+ bone marrow cells repopulating central autonomic nuclei

Authors: *Y. SUGIYAMA[1], G. FARACO[1], A. MORAGA[2], L. GARCIA BONILLA[1], J. ANRATHER[1], C. IADECOLA[1];
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Abstract: Trafficking of circulating bone marrow (BM)-derived cells into the hypothalamic paraventricular nucleus (PVN) has been implicated in critical autonomic functions, such as stress hormone responses (Neuron 65:94, 2010) and sympathetic activation during cardiac failure (Hypertension 55:652, 2010). Transplantation of GFP+ BM has been used extensively to localize BM-derived cells in the mouse brain. However, the regional distribution and the phenotypic characteristics of GFP+ cells in brain regions involved in autonomic regulation have not been clearly defined. We transplanted GFP+ BM into lethally irradiated C57Bl/6 mice (n=5/group) and examined phenotype and regional distribution of the cells 7 and 14 weeks later in brain sections by confocal microscopy. GFP+ cells were observed in brain regions involved in autonomic regulation, such as the PVN, nucleus tractus solitarius (NTS), area postrema (AP), and subfornical organ (SFO), but also in the somatosensory cortex. However, in PVN and NTS the number of cells increased markedly between 7 and 14 wks (e.g., PVN: 7 wks: 49±10; 14 wks: 210±45, $p<0.05$), whereas in cerebral cortex, SFO and AP the number of GFP+ cells did not change significantly (e.g., cortex: 7 wks: 106±20/section; 14 wks: 129±22/section, $p>0.05$). Next, we characterized GFP+ cells based on location, morphology and expression of myeloid markers. Two distinct populations were identified: (a) elongated cells, closely associated with blood vessels and expressing the perivascular macrophage marker CD206 (GFP+/CD206+) and (b) stellate cells with dendritic ramifications resembling microglia, not associated with blood vessels and expressing the microglia/macrophage marker Iba1 (GFP+/Iba1+). At 14 weeks, GFP+/CD206+ cells were most abundant in cortex, AP and SFO, whereas GFP+/Iba1+ cells were most abundant in PVN and NTS. The increase in GFP+ cells in PVN and NTS was entirely attributable to expansion of the GFP+/Iba1+ population (e.g., PVN: 7 wks: 21±7; 14 wks: 124±37). Furthermore, GFP+/CD206+ cells, but not GFP+/Iba1+ cells, were able to take up fluorescent dextran injected intracerebroventricularly, demonstrating a difference in phagocytic activity between the two groups. We conclude that there are fundamental regional and phenotypic differences in BM-derived cells repopulating the brain, non-phagocytic microglia-like cells predominating in the PVN and NTS, and phagocytic perivascular macrophages in neocortex and circumventricular organs (SFO, AP). It remains to be established whether these phenotypic differences are induced by regional molecular cues or reflect distinct homing programs intrinsic to BM-derived cells.

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Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 276.15/EEE14

Topic: E.02. Neuroimmunology

Title: Dendritic cell vaccine-induced immune activity in gliomas elicits ependymal gene expression and differentiation

Authors: *M. C. JHUN[1], N. YEAGER[1], R. CORDNER[2], A. RENTSENDORJ[1], A. MARDIROS[4], A. PANWAR[3], Y. HIRAKAWA[3], D. K. MORRIS-IRVIN[1], C. WANG[4], K. L. BLACK[1], C. J. WHEELER[1];
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Abstract: Glioblastoma Multiforme (GBM) is the most aggressive form of primary brain cancer. Even with aggressive surgical and chemical therapies, the overall prognosis is the most dismal of central nervous system cancers. In efforts to identify better treatment options, several subclasses of malignant gliomas are now distinguishable by global gene expression profiling, which can also identify tumor differentiation status. Microarray gene expression studies have identified differentiation profiles of tumor cells before and after Dendritic-Cell (DC) Vaccination. These profiles accompanied with fluorescence imaging of characteristic profile markers, track movement away from an astrocytic, mesenchymal profile after DC vaccination in both human GBM and mouse GL26 gliomas, towards a more ependymal-like profile. The decreased astrocytic and increased ependymal tumor gene expression taken together suggests heightened neuroepithelial differentiation. Furthermore, Fas-L deficient (Gld) mice and mice deficient for T cell functions showed evidence of increased invasive properties, while treatment with CD8 T-Cells, or prior exposure to CD8 T-Cells with temozolomide (TMZ) showed significantly similar decreased invasiveness. Dendritic-Cell Vaccination-elicited selection pressure may cause the ependymal-like differentiation which confers protection against immune predation of gliomas. Further identifying factors to perturb may possibly lead to development of longer times until tumor treatment-resistance resulting in longer survival for patients.

Disclosures: M.C. Jhun: None. **N. Yeager:** None. **R. Cordner:** None. **A. Rentsendorj:** None. **A. Mardiros:** None. **A. Panwar:** None. **Y. Hirakawa:** None. **D.K. Morris-Irvin:** None. **C. Wang:** None. **K.L. Black:** None. **C.J. Wheeler:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 276.16/EEE15

Topic: E.02. Neuroimmunology

Title: The CD200L-CD200R interaction is essential for the repair process following spinal cord injury

Authors: *M. COHEN, H. BEN-YEHUDA, M. SCHWARTZ;
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Abstract: The isolation of the central nervous system (CNS) from the periphery results in unique defense mechanisms that protect the CNS from uncontrolled inflammation. During homeostasis, the interaction between CD200L (OX2), expressed on neurons, and CD200R, expressed on resting microglia inside the CNS, is one of the most important axes to regulate microglial function, and delivers an inhibitory signal. Here, we probed the role of this interaction following spinal cord injury (SCI). Recovery of CD200-/- mice following SCI was impaired compared to wild type (WT) mice. CD200L expression by resident CNS cells, and not by the peripheral infiltrating cells, was essential for the repair process. On day 7 following SCI, CD200R was highly expressed by IB4+ activated microglia and macrophages, which were localized at the center of the lesion site. Moreover, while injured neurons down-regulated CD200L expression, CD31+ and CD34+ endothelial cells elevated its expression at the site of injury. CD200 deficiency in injured mice led to dysregulated organization of IBA1+ activated microglia compared to controlled localization of IBA1+ microglia in WT mice, which was also in close proximity to the CD200L+CD31+CD34+ endothelial cells. mRNA analysis of the cytokine milieu within the site of the injured spinal cords indicated that CD200L expression in the CNS might play a role in regulating the inflammatory response during the first 3 days following SCI. Furthermore, its deficiency seemed to affect the ability of the residing immune cells to express matrix metalloproteinases (MMPs), which have an important role in the degradation of the extracellular matrix following SCI. Thus, following SCI, CD200L expression by CNS endothelial cells compensates the down-regulation of its expression by neurons. Moreover, CD200L not only regulates inflammation, but also the induction of MMP expression.

Disclosures: M. Cohen: None. **H. Ben-Yehuda:** None. **M. Schwartz:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Topic: E.02. Neuroimmunology

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American Heart Association 09SDG2260983

Dana Foundation to ZG

Title: Quantitative proteomic analysis for the effects of aged garlic extracts on lipopolysaccharide-induced neuroinflammation in microglial cells

Authors: *Z. QU[1,2], H. ZHOU[1,2], D. L. NKHOLISE[1,2], V. V. MOSSINE[3], J. CUI[1,2], J. LI[4], J. CHENG[4], C. M. GREENLIEF[5], T. MAWHINNEY[3], P. N. BROWN[7], K. L. FRITSCHE[6], D. B. LUBAHN[3], G. Y. SUN[1,2,3], Z. GU[1,2];
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Abstract: Microglia are the resident immune effector cells in the central nervous system. Microglia can be activated by endotoxins, lipopolysaccharides (LPS) upon phagocytosis of invading bacteria, and produce pro-inflammatory radical mediators including nitric oxide (NO). Excess NO is thought to induce nitrosative stress and contribute to neuronal injuries leading to the progression of neurodegenerative diseases. There is a long-standing interest in botanicals as potential sources for the prevention of neuroinflammation and treatment of neurodegenerative diseases. Botanical compounds, aged garlic extract (AGE) and its active component D-fructose-L-arginine (FruArg), have been shown antioxidative activity. In this study, we investigated the effects of AGE and FruArg on LPS-induced neuroinflammatory response in immortalized murine microglial BV-2 cells by a quantitative proteomic approach. BV-2 cells were exposed to 100 ng/mL LPS for 20 hours in the presence or absence of 1% AGE or 3 mM FruArg. A Griess assay indicated both AGE and FruArg inhibited NO production in LPS-activated BV-2 cells, while no significant toxicity appeared in the cells as determined by MTT assay. Cell lysates of the samples (untreated, LPS-treated, LPS+AGE, LPS+FruArg) in biological triplicates were then labeled with CyDyes (using GE minimal labeling) and resolved by two dimensional difference

in-gel electrophoresis (2D-DIGE). Using Progenesis SameSpot software, we detected a total of 1,925 protein spots on the 2D-DIGE gels. Compared to LPS-treated conditions, 21 and 18 protein spots showed significant protein expression level changes (fold changes > 1.5, $p < 0.05$) after treatment with AGE and FruArg, respectively. Protein identification by liquid chromatography tandem mass spectrometry determined 24 proteins from the 21 spots and 20 proteins from the 18 spots. Ingenuity Pathway Analysis (IPA) with these proteins suggested oxidative stress and Nrf2-mediated antioxidative response were among the top toxicity and canonical pathways targeted by both AGE and FruArg. These results revealed the ability of AGE and FruArg to attenuate LPS-induced neuroinflammatary response in microglial cells.

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Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Topic: E.02. Neuroimmunology

Support: NRF Grant 2012008880

IPET Grant 110056-03-3-HD110

IPET Grant 112020-03-1-CG000

Title: Mitochondrial dynamics modulate the expression of pro-inflammatory mediators in microglial cells

Authors: *J. PARK[1], H. CHOI[2], D.-S. LEE[1];
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Abstract: Over-activation of microglia cells contributes to neurodegenerative processes. Recent evidence demonstrated that mitochondrial dynamics are an important constituent of cellular quality control and function.
In the present study, we found that LPS stimulation induced excessive mitochondrial fission in BV-2 microglia cells. Drp1-Ser637 phosphorylation as key regulator of mitochondrial fission was decreased. Furthermore, inhibition of LPS-induced mitochondrial fission by Mdivi-1, a

mitochondrial fission inhibitor, attenuated the production of pro-inflammatory mediators via reduced NF-κB, ERK, and p38 MAPK signaling, thereby suppressing mitochondrial fission-induced ROS production.
We therefore suggest that mitochondrial dynamics may regulate activation of microglia cells in brain.

Disclosures: J. Park: None. **H. Choi:** None. **D. Lee:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Topic: E.02. Neuroimmunology

Support: NIH Grant RO1AG-033028

Title: Active microglia are modulated by TGFb that is produced by IL-10 re-directed astrocytes

Authors: *D. M. NORDEN, A. M. FENN, A. DUGAN, J. P. GODBOUT;
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Abstract: Following immune challenge, active microglia in the aged brain produce exaggerated levels of both pro- and anti-inflammatory cytokines, including interleukin (IL)-10. Under normal conditions, IL-10 acts to resolve inflammatory processes within the CNS. High IL-10 production in the aged brain, however, did not correspond with reduced inflammation. We interpret these results to indicate that responsiveness to IL-10 is impaired in the aged brain. To begin to address this notion, we investigated the ability of IL-10 to regulate microglia and astrocytes using adult mice and series of cell culture experiments. In initial studies, expression of IL-10 receptor (IL-10R) was determined on astrocytes and microglia. While a subset of astrocytes expressed IL-10R, microglial expression of IL-10R was limited. Moreover, surface expression of IL-10R was increased on astrocytes, but not microglia, 24 h after immune challenge. Corresponding with these receptor results, primary cell culture experiments showed that astrocytes were more sensitive to the anti-inflammatory effects of IL-10 than microglia. For example, IL-10 stimulation of active primary astrocytes reduced the expression of inflammatory mediators (IL-1b, TNFa, IL-6). IL-10, however, had little effect on the pro-inflammatory profile of active microglia. To determine whether microglial activation could be indirectly modulated by IL-10 signaling in astrocytes, expression of anti-inflammatory cytokines induced by IL-10 treatment of astrocytes was determined. We show that activated astrocytes increased TGFb mRNA and protein expression which was significantly augmented in the presence of IL-10. Next, the ability

of TGFb to modulate microglia activation was investigated. In primary microglia, TGFb attenuated LPS induced expression of IL-1b and IL-6 and increased expression of two key regulatory receptors CX3CR1 and IL-4R. Using astrocyte-microglia co-culture systems, we confirmed that astrocytes mediate the anti-inflammatory effects of IL-10 on microglia via the production of TGFb. For instance, only when astrocytes were present did IL-10 stimulation result in lowered expression of IL-1b and increased expression of CX3CR1 and IL-4R. Importantly, all of these IL-10-astrocyte dependent effects on microglia were blocked by a TGFb inhibitor. Thus, we postulate that IL-10 re-directs active astrocytes to augment the production of TGFb, which in turn, attenuates microglial activation. Overall, these findings provide novel insight into the mechanisms by which astrocytes modulate microglia, and may provide new avenues to understand impaired regulation of microglia in the aged brain.

Disclosures: D.M. Norden: None. **A.M. Fenn:** None. **A. Dugan:** None. **J.P. Godbout:** None.

Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 276.20/EEE19

Topic: E.02. Neuroimmunology

Support: Charitable Hertie Foundation (PhD Stipend)

Title: Homeostatic and injury-induced microglial behavior in the aging brain

Authors: *J. K. HEFENDEHL[1,2], J. J. NEHER[2], R. SÜHS[2], S. KOHSAKA[3], A. SKODRAS[2], M. JUCKER[2];
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Abstract: Microglia cells are essential for brain homeostasis and have essential roles in neurodegenerative diseases. Because aging is the main risk factor for most neurodegenerative diseases, microglia aging may contribute to the susceptibility of the aged brain to dysfunction and neurodegeneration. Microglia aging in the past has been studied in postmortem brain tissue. Today we know, however, that microglia are highly dynamic surveying cells that continuously scan their environment every few hours and respond dynamically to disturbances in the brain (Nimmerjahn et al., Science 2005; Davalos et al., Nature Neuroscience 2005). Thus, postmortem microglia analysis alone is arguably not an appropriate method to assess microglia aging. In the present study we have analyzed microglia behavior in vivo in young adult (3 mo-old), adult (11-12 mo-old) and aged (26-27 mo-old) mice using in vivo 2-photon microscopy. Our results show

that surveying microglial cells in the neocortex exhibit age-related soma volume increase, shortening of processes, and loss of homogeneous tissue distribution. Furthermore, the baseline microglial process speed significantly decreased with age. While only a small population of microglia showed soma movement in adult mice, the microglia population with soma movement was drastically increased in aged mice. However, in response to tissue injury the dynamic microglial response was age-dependently diminished. These results provide novel insights into microglial behavior and indicate that microglial dysfunction in the aging brain may contribute to age-related cognitive decline and neurodegenerative diseases.

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Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Topic: E.02. Neuroimmunology

Support: Intramural grant 12643P

Title: Urban particulate matter selectively affects primary human neurons

Authors: K. MENDOZA[1], C. SIOUTAS[3], P. PAKBIN[3], *A. G. CAMPBELL[2];
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Abstract: Exposure to particulate matter (PM), found in urban air pollution, is considered a risk factor in many respiratory and cardiovascular diseases. More recently it has been speculated that PM exposure may also cause adverse effects in the brain. Although the exact mechanisms involved are unknown, enhancement in both oxidative and inflammatory responses has been reported. Since the main route of exposure to particulate matter is through inhalation, there is a potential for compounds to rapidly enter the brain through the cribriform plate of the ethmoid bone, resulting in direct activation of CNS inflammatory responses. The purpose of this study was to use normal human brain cells to assess cell type specific effects of PM exposure. We hypothesized that particles would activate microglial cells. Reactive oxygen species formation and the proinflammatory cytokine tumor necrosis factor alpha (TNF-α) were measured as markers of oxidative stress or inflammation respectively. Human microglia, neurons, and astrocytes were grown separately or as co-cultures and then exposed to PM collected from a site in Los Angeles. Surprisingly, the only human CNS cell type which was responsive to PM was

neuronal. Exposed cells showed a decrease in ROS formation but an increase in TNF-α production. This is contrary to what is seen in primary rodent CNS cells. Thus, the CNS response in normal human cells may be different compared to primary rodent cells. This is not surprising since a new report indicates that mouse models poorly reflect human inflammatory diseases. Thus the use of human cells may be more predictive than animal models in determining risk assessment of environmental factors.

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Poster

276. **Neuroimmunology: Microglia and Dendritic Cells**

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Topic: E.02. Neuroimmunology

Support: Pelotonia Idea Grant

Title: Microglia mediate chemotherapy induced neuroinflammation and cognitive deficits in female mice

Authors: *B. L. JARRETT[1], M. B. LUSTBERG[2], A. HINZEY[1], C. L. SHAPIRO[2], A. C. DEVRIES[1];
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Abstract: Although improvements in cancer detection and treatment have substantially increased the survival rate for breast cancer patients, the treatment regimens often impair quality of life. Chemotherapeutic drugs, particularly anthracyclines, have potentially toxic side effects in the brain affecting memory, processing time, and verbal fluency. More than 30% of patients undergoing chemotherapy for breast cancer report cognitive deficits during and after treatment. Despite the high incidence and significant impact on quality of life, little is known about the cause of the cognitive deficits and no effective treatment currently exists. Our hypothesis is that chemotherapy induces neuroinflammation and related cognitive impairment side effects that can be ameliorated though treatment with minocycline, an anti-inflammatory drug. Adult female BALB/C mice were ovariectomized and then two weeks later injected intravenous (IV) with a single dose of chemotherapy (45 mg/m^{2}doxorubicin + 450 mg/m^2 cyclophosphamide) or vehicle (n=10-12/group). The mice were trained for three trials per day across six days in the Barnes Maze, a well-characterized spatial memory task.
Both the chemotherapy-treated and vehicle-treated mice were able to acquire the task by the final

day of training. However, the chemotherapy-treated mice took significantly longer to find the escape box than the vehicle-treated mice on training days 2-5 ($p<0.05$). In contrast, there were no group differences in overall locomotor activity level, suggesting that the observed difference in maze performance was not due to fatigue. ($p>0.05$) In addition, chemotherapy increased proinflammatory cytokine expression (TNF-, IL-1, and IL-6) and altered neuronal morphology in the hippocampus ($p<0.05$), a region of the brain that is critical for spatial learning and memory. Likewise, microglia from chemotherapy-treated mice exhibited increased expression of MHCII and CD80 relative to vehicle-treated mice ($p<0.05$), thereby suggesting a proinflammatory phenotype.
A second cohort of mice was given minocycline (80 mg/kg/day) dissolved in the drinking water, or normal drinking water, before injection of chemo or saline. Chemo treated mice receiving minocycline improved learning and memory, similar to that of the control mice and also decreased proinflammatory and microglial activation markers. Together, these data suggest that the administration of doxorubicin and cyclophosphamide produce neuroinflammation, including microglia activation, and concomitant cognitive deficits in female mice and that these side effects can be eliminated with minocycline treatment.

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Poster

277. **Neuroimmunology: Behavioral Effects**

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Topic: E.02. Neuroimmunology

Support: Research Fund for the Control of Infectious Diseases from Food and Health Bureau (09080822)

HKU Alzheimer's Disease Research Network under Strategic Research Theme on Healthy Aging, Seed Funding for Basic Science Research (201211159058)

Title: Differential modulation of bacteria-induced sickness and inflammation by double stranded RNA-dependent protein kinase (PKR)

Authors: *D. C.-H. POON[1], Y. S. HO[1,4], C. K. M. LOK[1], S. M. C. LEUNG[1], K. CHIU[1], R. C. C. CHANG[1,2,3];
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Abstract: Sickness refers to a set of adaptive, physiological (e.g. fever) and behavioral (e.g. malaise, anorexia, social withdrawal, and fatigue) changes in response to systemic inflammatory insults, e.g. infection and injury. While these responses collectively serve to facilitate an organism to recover, increasing lines of evidence indicate that sickness, if exacerbated, may precipitate depression and delirium. Hence, it will be beneficial to discover suitable approaches to modulate sickness so as to maximize its benefits and minimize its side effects. As double-stranded RNA-dependent protein kinase (PKR) is key kinase regulating inflammation, the aim of this study is to investigate whether PKR also plays a regulatory role in sickness. PKR+/+ and PKR-/- mice were infected subcutaneously with live Escherichia coli (*E. coli*.) or vehicle. Sickness was assessed by monitoring fever, food consumption, burrowing, and open field activity for five days. Moreover, the brain and the liver were collected for quantitative polymerase chain reaction (qPCR) of the inflammatory markers interleukin-1β (IL-1β) and cyclooxygenase (COX-2).After being challenged by *E. coli*., PKR-/- mice developed prolonged fever (2-3 days) as compared to PKR+/+ mice (3-4 days). Moreover, PKR-/- mice showed greater hypophagia during the early phase of sickness (day 1-2), which was followed a more pronounced hyperphagia in the late phase of sickness (day 4-5). While both types of mice displayed similar decreases of burrowing activities, PKR-/- mice exhibited a greater reduction in open field activity than PKR+/+ mice. Furthermore, IL-1ß and COX2 in PKR +/+ and PKR-/- mice were differentially expressed in the brain and the liver following *E. coli.* challenge.It has been known that PKR participates in up-regulating inflammatory responses in immune cells, and that inflammatory factors such as IL-1β and COX-2 are key players in causing sickness during systemic inflammation. Our data is consistent with the literature that deficiency of PKR could lower the expression of systemic inflammatory markers after immune challenge. These findings shall (1) shed light on the suitability of PKR as a target to modulate sickness, and (2) stimulate us to refine the current concepts relating infection, systemic inflammation, and sickness.

Disclosures: D.C. Poon: None. **Y.S. Ho:** None. **C.K.M. Lok:** None. **S.M.C. Leung:** None. **K. Chiu:** None. **R.C.C. Chang:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 277.02/EEE23

Topic: E.02. Neuroimmunology

Support: Natural Sciences and Engineering, Research Council of Canada

Canadian Institute of Health Research

Title: Perturbation of the innate immune system in neurons leads to depression in mouse

Authors: *K. KEYHANIAN[1,3], X. ZHOU[3], N. R. PANDEY[3], Z. QIN[1,3], T. HO[2], K. WEN[4], A. F. R. STEWART[5], H.-H. CHEN[3];
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Abstract: Brain inflammation has been linked to chronic depression. Viral infection may act as a trigger preceding chronic depression. Half of patients who receive interferon alpha therapy to treat hepatitis infection develop depression. Interferon alpha is activated as part of the innate immune response to viral infection and this depends on the activity of the interferon regulatory factor transcription factors (IRF1, IRF3, IRF7). In contrast, IRF2 acts to suppress interferon alpha expression and limits the inflammatory response via the co-repressor IRF2-binding protein 2, IRF2BP2. To study the role of interferon alpha and inflammation on depression, we ablated IRF2BP2 in neurons of the forebrain. This leads to elevated interferon alpha expression. In behavioral studies, the sucrose preference test revealed a marked depression phenotype in these mice compared to littermate controls. Our studies further indicate that IRF2BP2 protein levels in neurons are regulated in response to metabolic perturbations and ischemia-reperfusion. The regulatory mechanisms that control IRF2BP2 protein levels are being elucidated. Disrupted expression of IRF2BP2 may affect the duration and extent of the inflammatory response and underlie chronic depression associated with metabolic and ischemic brain disorders.

Disclosures: K. Keyhanian: None. **X. Zhou:** None. **N.R. Pandey:** None. **Z. Qin:** None. **T. Ho:** None. **K. Wen:** None. **A.F.R. Stewart:** None. **H. Chen:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

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Åhlénsstiftelsen

Fredrik och Ingrid Thurings foundation

Svenska Läkaresällskapet

Foundation in memory of Lars Hierta

Title: Electroconvulsive therapy changes the plasma cytokine profile in patients with depressive disorder

Authors: *L. SCHWIELER[1], M. SAMUELSSON[2], H. LITWIN[1], S. ERHARDT[1];
[1]Karolinska Inst., Stockholm, Sweden; [2]Section of Psychiatry, Fac. of Hlth. Sci., Dept of Clin. and Exptl. Med., Linköping, Sweden

Abstract: Introduction
Depression is a severe, life-threatening illness exposing the patient to direct risks, such as suicide and lower quality of life. The pathogenesis of depression is still not fully understood but recent studies point to an involvement of the inflammatory system. In particular, activation of cytokines is suggested to play an important role in the pathophysiology. Electroconvulsive therapy (ECT) is regarded as an effective treatment of drug-resistant depression. The mechanism of action of ECT is unclear, although studies have investigated its effects on neurotransmitters and their receptors, neuropeptides, hormones, and neurotrophic factors. The aim of the present study is to investigate peripheral levels of cytokines in patients suffering from depression before and after treatment with ECT.
Methods:
Plasma cytokine concentrations (Interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, GM-CSF and INF-ϒ) were analyzed with electrochemiluminescence biosensor immunoassay (Meso Scale Discovery) in 27 patients with depression. The majority (n=23) of these patients had been treated with oral antidepressants but had not responded to drug treatment, and four were treatment naïve. Diagnosis was based on clinical interviews by a senior psychiatrist and the severity of symptoms was rated with Brief Psychiatric Rating scale (BPRS) and global assessment of functioning (GAF). To estimate the severity of depression during the course of the ECT treatments, the psychiatrist performed a Montgomery-Åsberg Depression Rating Scale (MADRS) scoring before the first and after the third ECT administration.
All patients where treated with right unilateral ECT treatments after at least 6 h of fasting. Plasma samples were collected before first ECT (n=27), after third (n=23) and sixth (n=12) ECT. All samples where stored at − 70 °C until analysis.
Results:
Preliminary data show that plasma levels of IL-6, IL-8 and TNF-α increase after ECT treatment with the most tangible effect after the sixth ECT. The mean MADRS score for all patients was 36.9± 1.5 before the ECT therapy started and 19.5 ± 2.0 after three ECT administrations. When data are subgrouped according to the treatment outcome into responders (MADRS score ≤ 15; n = 7) and non-responders (MADRS score > 15; n = 15) the effects on plasma cytokines are even

more pronounced, especially with regard to IL-6.
Conclusion:
The present results suggest that ECT changes the plasma levels of various cytokines in patients with depression. Therefore, cytokines might play a specific role within the treatment and pathogenesis of affective disorder.

Disclosures: L. Schwieler: None. **M. Samuelsson:** None. **H. Litwin:** None. **S. Erhardt:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

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Program#/Poster#: 277.04/EEE25

Topic: E.02. Neuroimmunology

Title: L. reuteri-induced modulation of behavior in C57BL/6J mice

Authors: *M. J. EIMERBRINK, E. E. JOHNSON, G. W. BOEHM;
Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: There is a well-established communicative link between the immune system and central nervous system by which each has regulatory influence over the other. This bidirectional communication facilitates adaptive behaviors during peripheral immune activation commonly known as sickness behaviors. Sickness behaviors include a variety of responses, but there are specific behaviors that are mediated by an increase in pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α. Interestingly, recent studies also indicate that the microbiota of the gut can interact with the immune system to indirectly exert influence on an organism's behavior. The purpose of this research was to manipulate the gut microbiota to produce alterations in the immune system of C57 BL/6J mice that protect against the immune response to LPS. To achieve this goal, we utilized a treatment of the probiotic Lactobacillus reuteri to buffer the inflammatory response to LPS. Additionally, we used direct injections of IL-10 as a positive control against LPS-induced proinflammatory cytokine expression to test the efficacy of both treatments in rescuing burrowing behavior, as well as learning and memory. We hypothesize that behavioral changes will also be corroborated with subsequent decreases in hippocampal decreases in pro-inflammatory cytokines. In summation, this research looked to identify the effects of L. reuteri and IL-10 on LPS-induced sickness behavior and memory alteration.

Disclosures: M.J. Eimerbrink: None. **E.E. Johnson:** None. **G.W. Boehm:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

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Program#/Poster#: 277.05/EEE26

Topic: E.02. Neuroimmunology

Support: NIH 4R00AG040194-02

Title: Role of inflammation in high-fat diet-induced cognitive deficits and alterations in hippocampal neurogenesis

Authors: K. M. BAUMGARNER, C. DIAZ, A. JONES, *R. A. KOHMAN;
Psychology, Univ. of North Carolina Wilmington, Wilmington, NC

Abstract: Obesity is a risk factor for several life-threatening diseases and neurological disorders including cardiovascular disease, diabetes, metabolic syndrome, dementia and Alzheimer's disease. One factor that may contribute to the increased risk for these conditions is the development of chronic inflammation. The current study evaluated whether consumption of a high-fat diet affects cognitive performance, measures of neural plasticity, and alters the inflammatory response to an immune challenge. Two experiments were conducted using adult male C57BL/6J mice that were fed ad libitum either a high-fat diet (HFD; 60% fat) or a control diet (CD; 10% fat) for 2 or 5 months. Initial results indicate that consumption of the HFD impaired acquisition of a spatial learning task relative to mice consuming the CD. Further, in response to the endotoxin lipopolysaccharide (LPS) mice fed the HFD showed higher splenic and hippocampal levels of the proinflammatory cytokine interleukin-1β relative to mice fed the CD. Additional work is underway to assess the impact of consuming the HFD on LPS-induced alterations in hippocampal neurogenesis, as measured by new cell survival and cellular differentiation. We hypothesize that consumption of the HFD in combination with an immune challenge will further reduce new cell survival relative to each treatment alone. Further, the lab is currently investigating the potential effects of consuming a HFD on the behavioral response to LPS administration as well as additional measures of immune activation within the brain. Presently, the data indicate that HFD consumption can disrupt cognitive function and exaggerate the inflammatory response within both the periphery and the brain.

Disclosures: K.M. Baumgarner: None. **C. Diaz:** None. **A. Jones:** None. **R.A. Kohman:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 277.06/FFF1

Topic: E.02. Neuroimmunology

Title: More pain than gain with chronic codeine: The first evidence of codeine-induced hyperalgesia

Authors: *J. L. JOHNSON[1], M. R. HUTCHINSON[2], D. B. WILLIAMS[3], P. ROLAN[1,4]; [1]Discipline of Pharmacol., [2]Discipline of Physiol., Univ. of Adelaide, Adelaide, Australia; [3]Sch. of Pharm. and Med. Sci., Univ. of South Australia, Adelaide, Australia; [4]Pain and Anaesthesia Res. Clin., Adelaide, Australia

Abstract: Background: Chronic opioid therapy is associated with opioid-induced hyperalgesia and allodynia, paradoxically increased pain sensitivity. Codeine is a widely used opioid that relies upon partial metabolism (approximately 10%) to morphine to elicit analgesia, owing to codeine's poor opioid receptor affinity. Chronic morphine exposure can exacerbate pain by activating the spinal innate immune system in a toll-like receptor-4 (TLR4) dependent manner. In silico docking simulations indicate codeine also docks to TLR4, suggesting potential to induce TLR4-dependent pain enhancement independent of conversion to morphine or opioid receptor activity.
Hypothesis: Codeine and morphine will cause TLR4-dependent hyperalgesia/allodynia that is independent of their opioid receptor-dependent analgesic rank potency.
Aims: Compare chronic codeine and morphine induced hyperalgesia/allodynia in mice and investigate mechanisms contributing to increased pain sensitivity.
Methods: Hyperalgesia and allodynia were assessed using hot plate and von Frey tests respectively, at baseline, day 3 and day 5 in male BALB/c mice receiving equimolar codeine 21 mg/kg (n=8), morphine 20mg/kg (n=8) or saline (n=8), twice daily (i.p.). The impact of Interleukin-1b on pain behavior was assessed on day 5 with the administration of interleukin-1 receptor antagonist (IL-1RA) prior to testing.
Results: Hot plate latency (s) was reduced, in codeine (-9.5, CI:4.7-14.3) and morphine (-7.3, CI:2.3-12.3) groups compared to saline at day 5. An increase in von Frey paw withdrawals (out of 10), only reached significance in the morphine group on day 5 (2.3, CI: 0.6-4). IL-1RA reversed codeine- (8.6, CI:5.1-12.2 and -2.1, CL:0.6- 3.6) and morphine-induced (10.4, CI:6.9-14 and -1.8, CI: 0.2-3.3) changes in hot plate latency and paw withdrawals, respectively.
Conclusions: Equimolar doses of codeine and morphine induce similar degrees of Interleuklin-1b-dependent hyperalgesia and allodynia, suggesting codeine does not rely upon conversion to morphine to increase sensitivity to noxious stimuli, emphasising the non-opioid receptor dependent nature of opioid-induced hyperalgesia. Ongoing studies examining priming with partial nerve injury, TLR4 knockout mice, a glial attenuator (ibudilast), and proteomic analysis

of collected spinal cord samples will explore the underlying cellular and molecular mechanisms further.

Disclosures: J.L. Johnson: None. **M.R. Hutchinson:** None. **D.B. Williams:** None. **P. Rolan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-holder of a provisional patent for the use of ibudilast in the treatment of medication overuse headache.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 277.07/FFF2

Topic: E.02. Neuroimmunology

Support: NIH Grant R01-MH09127

Hogg Foundation for Mental Health

Title: Amyloid-beta induces a neuroinflammatory response and indoleamine 2,3-dioxygenase-dependent neuropsychiatric-like behaviors in mice

Authors: *J. M. PARROTT, L. REDUS, A. GREEN, J. C. O'CONNOR;
Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: Retrospective studies indicate that about one-third of Alzheimer's disease (AD) patients were previously diagnosed with depression prior to developing AD symptomology. Amyloid-beta (Aβ) plaques and neurofibrillary tangles begin to accumulate long before overt cognitive symptoms develop in humans, so early accumulation of Aβ may stimulate a neuroinflammatory response and subsequent neuropsychiatric symptoms.
Here, we sought to determine if acute administration of Aβ42 would induce a neuroinflammatory response and precipitate depressive-like behaviors in mice. Using the young (2-month) "triple-transgenic" mouse model of AD (3xTg-AD) with no overt cognitive deficits, Aβ42 levels and the expression of several neuroinflammatory markers in the hippocampus, striatum, frontal cortex and amygdalae were measured. Compared to age-matched wild-type (WT) control mice, 3xTg-AD mice had detectable increases in Aβ42 in the hippocampus and amygdala. Further, in all of the brain regions assessed, 3xTg-AD mice exhibited a marked neuroinflammatory response, characterized by up-regualtion of pro-inflammatory cytokines and kynurenine pathway enzymes. To determine whether Aβ42 could directly induces a similar neuroinflammatory response, WT mice were administered 400pmol Aβ42, or vehicle, directly into the lateral ventricles, followed

by assessment of neuropsychiatric-like behaviors and tissue collection for analysis of neuroinflammatory status. Three days post-injections, Aβ42-treated mice exhibited a significant reduction in sucrose preference. After 7d, Aβ42 administration caused mice to spend less time in the central area of an open field and more time floating during the forced swim test. Further, an increase in pro-inflammatory cytokines and kynurenine pathway enzymes was present at 1 and 3 days post Aβ42 infusion.
To determine whether increased kynurenine pathway metabolism mediates the behavioral effects of acute Aβ42, indoleamine 2,3-dioxygenase (IDO) deficient mice, mice pretreated with the competitive IDO inhibitor, or WT vehicle mice received intracerebroventricular infusion with Aβ42 or vehicle. Interestingly, both genetic and pharmacological inhibition of IDO protected mice from the Aβ-induced decrease in sucrose preference, increase in central area duration, and increase in floating behavior observed in the forced swim test. Together, these data suggest that very early AD pathogenesis may drive comorbid depressive symptoms in an IDO-dependent manner.

Disclosures: J.M. Parrott: None. **L. Redus:** None. **A. Green:** None. **J.C. O'Connor:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

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Topic: E.02. Neuroimmunology

Support: PSC CUNY Grant 65379-00-43 to CFH

CUNY CIRG 1937 to CFH & CP

Research Centers in Minority Institutions Award RR-03037 from the NCRR to Hunter College

Title: Effects of mold exposure on anxiety, conditioned fear, motor performance and spatial memory

Authors: *C. F. HARDING[1,2], K. DENISOVA[1], T. DESAI[3], R. DESTEFANO[4], T. ROA[5], E. SHTRIDLER[1], C. PYTTE[2,6];
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Abstract: The common estimate is that approximately 40% of American buildings are moldy. Exposure to mold can cause neurological problems, increased anxiety, and cognitive deficits. However, no animal research has been published examining how mold exposure causes these problems. We developed a mouse model to determine how mold exposure can lead to neurobehavioral dysfunction. Our basic hypothesis is that mold inhalation, like bacterial infection, activates an innate immune response triggering sickness behavior with resultant behavioral dysfunction. There is controversy over whether only toxic molds, like *Stachybotrys chartarum*, can cause health problems, or if exposure to skeletal elements common to all molds is sufficient. We therefore compared mice treated with 1) intact, toxic *Stachybotrys* spores (IN), 2) extracted *Stachybotrys* spores that had their toxins removed and proteins denatured (EX), or 3) vehicle (VEH). Mice were treated with relatively low spore doses 3X per week. Three weeks of treatment led to significant increases in anxiety as measured by the elevated plus maze among EX mice compared to both VEH and IN mice. EX mice had a significantly lower percentage of open arm entries, percent duration in the open arms, and percent distance traveled in the open arms. All groups performed similarly on the training trial of an auditory-cued conditioned fear test. However, IN mice moved significantly less than VEH mice on the auditory test a day later, suggesting they were more fearful. Among mice that learned to find the platform in the Morris water maze after four visible platform training trials, IN mice took slightly longer to reach the hidden platform the following day and their paths to the platform were longer. Results of a subsequent probe trial suggested that IN mice were using a thigmotactic, rather than a spatial strategy to find the platform. After six weeks of mold treatment, IN mice also fell off the rotorod significantly earlier than VEH mice. These data indicate that mold exposure increases both general anxiety and fearful behavior, alters learning strategies, and interferes with motor performance. Contrary to popular opinion, skeletal elements are sufficient to elicit adverse effects. Increased anxiety, increased fearfulness, deficits in learning and memory, and deficits in motor performance have all been documented in mold-exposed patients. Other studies from our laboratories suggest that these problems are mediated by mold-induced microglial activation and resulting brain inflammation.

Disclosures: C.F. Harding: None. **T. Desai:** None. **R. DeStefano:** None. **T. Roa:** None. **E. Shtridler:** None. **C. Pytte:** None. **K. Denisova:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

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Topic: E.02. Neuroimmunology

Support: PSC CUNY Grant 65379-00-43

CUNY CIRG 1937

Research Centers in Minority Institutions Award RR-03037

Title: Repeated exposure to environmental mold increases cytokine interleukin-1β expression in the mouse hippocampus

Authors: *L. BLACHORSKY[1,2], K. PAGE[3], G. MANES[4], C. HARDING[4,3], C. PYTTE[3,1]; [1]Psychology, Queens College, CUNY, Flushing, NY; [2]Macaulay Honors Col., New York, NY; [3]Behavioral and Cognitive Neurosci. Doctoral Program, CUNY, New York, NY; [4]Psychology, Hunter College, CUNY, New York, NY

Abstract: Prolonged exposure to indoor mold can cause severe cognitive impairment, anxiety, and depression. However, the neural mechanisms underlying these symptoms have not been identified. Towards this aim, we have developed a mouse model to determine how inhaled mold spores affect the brain, leading to neural damage and cognitive and affective impairment. Our work focuses on the toxic "black" mold *Stachybotrys chartarum*, which is a common contaminant of damp and water-damaged buildings. Previous work from our labs has determined that repeated exposure to spores (3x per week for 3 weeks) caused striking deficits in hippocampal memory and increased anxiety compared to controls. Here we tested the hypothesis that these effects are mediated by central inflammation. Adult male C57BL/6 mice were given intranasal instillations of low doses of intact mold spores (IN), extracted spore skeletons (EX), or saline vehicle (VEH) 3x per week for 6 weeks. Extracted *Stachybotrys* spores have had their toxins remove and proteins denatured in order to test effects of exposure to non-toxic spore elements common to all molds. Moreover, extracted spore elements remain after killing mold in buildings with commercial products. Mice were tested with behavioral tests beginning at week 3, and were anesthetized and perfused after 6 weeks of treatment. Brains were processed for immunohistochemistry to label the pro-inflammatory cytokine Interleukin-1β (IL-1β) which is expressed primarily by microglia. Numbers of cells expressing IL-1β, and numbers of IL-1β - positive cellular processes, per area sampled were quantified throughout the CA1 and the whole hippocampus. We found significant differences in numbers of IL-1β-expressing cells and processes between IN, EX, and VEH groups in both the CA1 and throughout the entire hippocampus (ANOVA, $p<0.05$). On all measures, IN mice showed significantly more IL-1β expression than VEH mice (Tukey's posthoc test) while EX groups had intermediate values. These findings support our model that cognitive and affective impairment following repeated or prolonged exposure to mold triggers an innate immune response leading to central inflammation.

Disclosures: L. Blachorsky: None. **K. Page:** None. **G. Manes:** None. **C. Harding:** None. **C. Pytte:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

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CUNY CIRG 1937 to CH & CP

TRADA43687 (CP)

Research Centers in Minority Institutions Award RR-03037 from the NCRR to Hunter College

Title: Environmental mold decreases new neuron survival in the adult mouse hippocampus

Authors: *E. NORMAND[1,2], D. BEECH[5], S. RIBEIRO[5], K. PAGE[6], C. F. HARDING[3,7], R. DESTEFANO[1,4], D. MORRIS[2], C. PYTTE[5,6];
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Abstract: Indoor mold is known to cause systemic health problems and sometimes severe learning and memory impairments. To understand the effects that mold may have on the central nervous system, we have developed a mouse model to study neural and behavioral consequences of exposure to *Stachybotrys chartarum*, a species of "black" mold found in water-damaged buildings. *Stachybotrys* is a toxin-producing mold and it is controversial whether the toxins per se cause cognitive problems or whether basic components of the spore found in all molds are sufficient to trigger cognitive deficits. In our standard protocol, C57BL/6 mice are exposed to low doses of intact spores (IN), extracted spore casings (EX) in which the intact spores were treated with ethanol to remove toxins and denature proteins, or saline vehicle as a control (VEH). In previous work, we found that mice treated with EX spores or IN spores showed equivalent impairment of hippocampal-dependent learning and memory. We then tested the hypothesis that deficits in hippocampal-dependent tasks may be due to decreased survival of new neurons in the dentate gyrus. Interestingly, we found a decrease in doublecortin (DCX)-expressing young neurons in the dentate gyrus only in EX treated animals, suggesting that IN spores are acting via a different mechanism, or perhaps impact a different neuronal cohort, than EX spores. DCX is

expressed in post-mitotic migratory and young neurons up to about 1 month of age. Here we quantified new neuron survival of older neurons in IN, EX, and VEH treated groups. Adult male C57BL/6 mice were intranasally exposed to spores or vehicle 3x per week for 6 weeks. The mice were injected with BrdU (2 x day, 4 days) 34-37 days before perfusion to label mitotically active cells and then underwent behavioral testing to assess hippocampal-dependent learning and memory. To quantify new neurons in the hippocampus, cells containing BrdU and NeuN, a marker of mature neurons, were labeled using immunohistochemistry. We found a significant difference across groups (ANOVA, $p=0.02$). Moreover, mice treated with IN spores showed fewer new neurons than the VEH-treated group (Tukey's post hoc, $p<0.05$). The EX group was not significantly different from IN or VEH groups. Together, these results suggest that EX spores lead to decreased numbers of new neurons less than one month of age whereas IN spores result in decreased survival of neurons in a cohort 32-37 days of age. These findings are consistent with the hypothesis that EX and IN spores have different effects, or different time courses of effects, following exposure; both lead to decreased survival of new neurons and hippocampal learning and memory deficits.

Disclosures: E. Normand: None. **D. Beech:** None. **S. Ribeiro:** None. **K. Page:** None. **C.F. Harding:** None. **R. DeStefano:** None. **D. Morris:** None. **C. Pytte:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 277.11/FFF6

Topic: E.02. Neuroimmunology

Title: Exercise alters the adaptive immune profile in mice

Authors: K. POINSATTE[1], A. MCPARTLIN[1], A. J. M. MEEUWISSEN[1], D. CHEN[1], E. SHUBEL[1], X. KONG[1], N. MONSON[1], R. ZHANG[2], *A. M. STOWE[1];
[1]Neurol. and Neurotherapeutics, [2]Intrnl. Med., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Objective: Exercise provides neuroprotection from stroke-induced injury, as demonstrated in previous animal studies. Clinically, patients with high physical activity prior to stroke present with milder strokes and decreased deficits. However, the mechanisms for this protection remain unclear. This study used a voluntary exercise protocol to test the hypothesis that exercise induces adaptive immune mechanisms, which may be protective prior to stroke

onset.
Materials/Methods: Male Swiss Webster mice (6-12 wks old) were individually housed in cages with monitored running wheels (n=11) or locked running wheels (i.e. sedentary controls; n=11). Following three weeks of exercise, leukocytes were isolated from peripheral blood and spleen, stained with antibodies, and profiled on a BD-FACS Aria flow cytometer, while brains were collected for quantitative rt-PCR. Student's t-test or linear regression analysis determined significance ($p<0.05$). Additionally, sorted CD19+ B cells were isolated from spleen by flow cytometry (n=5/group), analyzed on an Illumina MouseWG-6 V2 Bead Chip, and final gene pathways determined using Ingenuity Pathway Analysis (IPA).
Results: Voluntary exercise decreased CD4 T cell representation ($p=0.05$) in the peripheral blood, with no other differential effect on CD8 T cells, neutrophils, B cells, or monocytes in the blood or spleen when compared to sedentary controls. Average weekly individual wheel rotations, however, were inversely proportional to the number of leukocytes ($p=0.06$), CD8 T cells ($p<0.05$), neutrophils ($p<0.05$) and neutrophil representations ($p<0.01$) in the spleen. Only B cells increased representation ($p<0.01$) in mice with higher levels of exercise. Mice with sustained levels of exercise over 3 wks also exhibited a unique clustering pattern in the CD19+ B cell microarray data. Microarray showed a significant upregulation of 1844 genes and downregulation of 1333 genes in the resident splenic B cells. Exercise upregulated genes associated with increased B cell maturation, generation and differentiation, with simultaneous downregulation of genes responsible for apoptosis and B cell death.
Conclusion: Three weeks of voluntary exercise in mice results in a change in the immune profile with enhanced expression of a unique, exercise-induced B cell phenotype. Downregulation of neutrophils and cytotoxic T cells also suggests that this alteration in immunity is anti-inflammatory. Further studies will determine the significance of these immune adaptations and their role in decreased neurovascular injury and functional deficits following stroke.

Disclosures: K. Poinsatte: None. **A. McPartlin:** None. **A.J.M. Meeuwissen:** None. **D. Chen:** None. **E. Shubel:** None. **X. Kong:** None. **N. Monson:** None. **R. Zhang:** None. **A.M. Stowe:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

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Program#/Poster#: 277.12/FFF7

Topic: E.02. Neuroimmunology

Support: NIMH93459

Title: Sex differences in hippocampal cytokine expression and signaling after myocardial infarction

Authors: *N. C. TRONSON, I. C. SPEIRS;
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Abstract: Activation of the innate immune system by chronic illness or injury is correlated with depression, anxiety and PTSD. In patients of heart attack (myocardial infarction, MI), rates of major depression and PTSD diagnoses are two- and three-fold higher, respectively, than the population at large. Consistent with other patients of PTSD and depression, post-MI incidence of these disorders is correlated with increased levels of circulating pro- inflammatory cytokines. In addition, women are more susceptible to both PTSD and depression. The effects of systemic inflammation in causing cytokine expression in the brain, and the role of their intracellular signaling cascades on memory and depression-like behavior in females and males, remains unknown. In this study, we used a surgical model of myocardial infarction in mice to investigate the role of sustained peripheral inflammation on sex differences in learning, memory and depression-like behaviors. Multiplex analysis of cytokines specifically within the hippocampus, and determination of JAK/STAT signal transduction was determined in male and female mice in the months after MI. These changes were correlated with alterations in memory and depression like behavior. Here, male, but not female mice exhibited enhanced fear conditioning soon after MI. In contrast, both males and females showed impaired memory and depression-like behavior at later time points. Thus we demonstrate differential cytokine expression and JAK/STAT signaling in the hippocampus in males and females, and determined their role in altered memory and affect. These findings suggest cytokine-dependent signaling in the brain after systemic inflammation as a candidate mechanism for susceptibility to, or development of depression, PTSD, and cognitive impairments.

Disclosures: N.C. Tronson: None. **I.C. Speirs:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 277.13/FFF8

Topic: E.02. Neuroimmunology

Support: SFB 854

Title: Very late antigen-4 mediated neutrophil invasion into ischemic brain leads to dynamic interactions with microglia and massive functional impairment

Authors: M. RIEK-BURCHARDT[1], J. NEUMANN[3], J. HERZ[4], *K. G. REYMANN[2,5], M. GUNZER[6];
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Abstract: The pathophysiology of ischemic stroke resulting from the occlusion of arterial vessels which leads to irreversible neuronal cell injury is further aggravated by invading peripheral immune cells. Recently, the common concept that early brain infiltrates are mainly neutrophil granulocytes is controversially discussed. However, all these studies are based solely on histological and flow cytometric analysis of ischemic brain tissue. But the cellular dynamics of neutrophil entry and the direct consequences for the affected brain area have remained unclear. We show by intravital two-photon microscopy in mice that neutrophils immediately recognize endothelial activation in stroke-related brain areas and start rolling and firm adhesion on such sites. We also show the extravasation into the impaired brain parenchyma and that microglia within the brain recognizes both, endothelial damage and neutrophil invasion. In a cooperative manner they form membrane processes to physically shield activated endothelia and trap infiltrating neutrophils. Further we demonstrate that the systemic blockade of Very-late-antigen 4 (VLA-4) effectively inhibits endothelial interaction as well as brain entry of neutrophils, which reduces behavioral impairmentsCollectively these data shows, that the anti VLA-4 treatment is neuroprotective and a critical receptor for neutrophil entry into the inflamed brain in the first 3 days after stroke.

Disclosures: M. Riek-Burchardt: None. **J. Neumann:** None. **K.G. Reymann:** None. **J. Herz:** None. **M. Gunzer:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 277.14/FFF9

Topic: E.02. Neuroimmunology

Support: NIH Grant MH 084980

Title: Long-term consequences of early life stress on immune function in rhesus macaques

Authors: *P. J. PIERRE[1], C. L. COE[2], A. J. BENNETT[2];
[2]Psychology, [1]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Human and animal studies demonstrate many profound and long-lasting negative health consequences of early life stress experiences (ELS), including those that disrupt social attachment relationships. Little is known, however, about the specific biological mechanisms by which these experiences alter individual's health across the lifespan. In this study, we used a well-validated nonhuman primate model of ELS (nursery-reared monkeys, NR) to assess the effects of infant experience on health in middle-age. Nursery-reared (NR) nonhuman primates exhibit robust alterations in many aspects of behavior and physiology that parallel those observed in humans who experience early life adversity. These include significant differences in immune function and health; however, little is known about the persistence of these effects into middle and older adulthood in nonhuman primates. This study evaluated the consequences of early differential rearing in multiple aspects of immune function in twelve sub-adult and twelve middle-aged male rhesus macaques, with six NR and six mother-reared (MR) monkeys in each age group . Blood samples were collected from animals lightly anesthetized for routine clinical health examinations. Samples were analyzed to provide white blood cell counts, platelet counts, mononuclear cell counts, number of lymphocytes and neutrophils. Cytokine concentrations, including interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF), were assayed in whole blood cell culture under both unstimulated conditions and in response to stimulation with LPS (E. coli derived). Key measures of immune function were significantly affected by early rearing experience in both sub-adult and adult monkeys. NR animals had lower neutrophil counts and significantly increased IL-6 concentrations in response to LPS challenge. The groups did not differ in cytokine concentrations of unstimulated cells. Age also influenced multiple immune measures, with increased white blood cell counts, increased percentage of lymphocytes and neutrophils, and decreased IL-8 concentrations. Together these results demonstrate that the effects of early social experience can have persistent effects on immune function into the middle-life period and in absence of subsequent differences in diet, medical care, or other environmental factors.

Disclosures: P.J. Pierre: None. **C.L. Coe:** None. **A.J. Bennett:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

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Program#/Poster#: 277.15/FFF10

Topic: E.02. Neuroimmunology

Support: NIH R01 MH082930

Title: Acute neuroinflammation disrupts context-object discrimination but not novel object recognition

Authors: *J. CZERNIAWSKI[1], J. F. GUZOWSKI[2];
[1]Neurobio. & Behavior, [2]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Neuroinflammation is now widely implicated in the deleterious effects on neuronal function due to aging, disease, and trauma. In the event of immune system activation, there is a robust and transient increase of immune molecules, such as cytokines and chemokines, in the brain. Emerging data indicate that cytokines, such as IL-1β, IL-6, and TNF-α, can affect synaptic plasticity and learning processes. The hippocampus in particular appears to be vulnerable since the induction of an immune response in rodents tends to disrupt hippocampus-dependent tasks such as the Morris water maze and contextual fear conditioning, while leaving hippocampus-independent tasks such as the cued version of the water maze or auditory fear conditioning intact. However, whereas most of these studies have examined the effect of immune challenge on the acquisition or consolidation of memories, humans with a variety of neuroimmune disorders can also have problems in memory retrieval. Therefore it is important to have a behavioral paradigm that can assess the impact of neuroinflammation on retrieval in order to develop potential therapeutic agents. We have implemented the use of novel object recognition (NOR) and context-object discrimination (COD) tasks in order to assess the effects of acute neuroinflammation on retrieval. Both of these paradigms exploit the animals' natural tendency to explore novelty but NOR tests the ability to remember a previously experienced object, whereas COD tests the ability to recognize that a previously experienced object is in a different context. Importantly, COD is widely considered to be dependent on the hippocampus whereas NOR is not. Male Sprague Dawley rats were trained in COD or NOR for two days, followed by testing on the third day. Subjects received a low-dose (100ug/kg) of lipopolysaccharide (LPS) systemically 6 hours prior to testing, a timepoint during which we observe peak levels of IL-1β, IL-6, and TNF-α in dorsal hippocampus. Subjects that received systemic LPS injections preferentially explored a novel object similar to controls. However, unlike controls, subjects injected with LPS did not exhibit greater exploration of a familiar object in a different context during COD testing. There no were differences in total exploration between LPS and control subjects for either experiment. These data indicate that acute neuroinflammation impaired the retrieval of context-object associations. Subsequent studies will use Arc/Homer catFISH in order to assess the effect of LPS administration on neural circuit activity in these tasks and explore potential therapeutic agents to block the deleterious effect of LPS on cognitive processes.

Disclosures: J. Czerniawski: None. **J.F. Guzowski:** None.

Poster

277. Neuroimmunology: Behavioral Effects

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 277.16/FFF11

Topic: E.02. Neuroimmunology

Title: Antipsychotic potential of thalidomide in immune/inflammatory model for schizophrenia

Authors: *M. MIZUNO[1], H. NAWA[2], K.-I. NAGATA[1];
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Abstract: Most of conventional antipsychotic drugs target monoamine receptors such as dopamine D2 receptors. In the present research, we aimed at developing a new class of antipsychotic drugs using the animal models for schizophrenia, which were established with maternal immune activation. We challenged pregnant mice and rats with bacterial lipopolysaccharides(LPS). At post-pubertal stage, these animals exhibited behavioral abnormalities in prepulse inhibition and social interaction. We examined the antipsychotic actions of immunomodulatory agents, thalidomide, which are known to attenuate inflammatory cytokine signaling. Subchronic p.o. administration of thalidomide for a week significantly ameliorated deficits of social interaction and prepulse inhibition in the animal model established with maternal LPS challenge. However, thalidomide alone did not affect weight gain and locomotor activity, suggesting no apparent side effects. Currently we are investigating the behavioral effects of thalidomide in these animal models. These results suggest that immunomodulatory or anti-inflammatory agents may be a new class of antipsychotic drugs for schizophrenia.

Disclosures: M. Mizuno: None. **H. Nawa:** None. **K. Nagata:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 277.17/FFF12

Topic: E.02. Neuroimmunology

Support: Bristol-Myers Squibb Company, USA

Title: Bacillus Calmette-Guérin vaccine-induced depression-like phenotype in mice: A selective serotonin reuptake inhibitor-resistant model

Authors: *V. K. KUCHIBHOTLA[1], A. RUDRA[1], M. V. SREEDHARA[1], S. SINGH[2], E. DURGA SHIVA PRASAD[1], M. LAL DAS[1], M. SENTHIL KUMAR[3], R. YADAV[3], R. K. TRIVEDI[3], S. N. PATTIPATI[1], L. BRISTOW[4], R. VIKRAMADITHYAN[1];
[1]Neuroscience, Biology(In-vivo), [2]Immunology, Biology(In-vivo), [3]Pharmaceut. Candidate Optimization, Biocon Bristol-Myers Squibb Res. Center, Syngene Intl. Limited, Bangalore, India; [4]Neurosci. Biol., Res. & Development, Bristol-Myers Squibb Co., Wallingford, CT

Abstract:
Major depressive disorder (MDD) is one of the leading causes of disability worldwide associated with persistent feelings of sadness, depressed mood, anhedonia and suicidal thoughts. In addition to the traditional hypothesis of monoamine deficiency, inflammatory responses are emerging as potential contributors towards the development of
depression symptoms in humans. Earlier reports indicated that a single injection of *Bacillus Calmette-Guerin* (BCG) vaccine results in a brief period of serum sickness followed by a long lasting depression-like phenotype in mice; however the responsiveness of BCG-induced depressive behavior to pharmacological treatment has not been extensively characterized. In the present study we induced depression-like behaviors in male BALB/c mice by administering the BCG-vaccine at a dose of 10^9 cells/mouse, i.p. A single injection of BCG-vaccine produced an inflammatory response including elevation of plasma IL-6 and IFN-gamma levels 24-hours after administration. We also observed a robust decrease in body weight and a reduction in locomotor activity and voluntary wheel running in BCG-treated mice compared to saline-treated
animals. Time course analysis showed a gradual recovery from serum sickness followed by the development of a significant depression-like phenotype, namely increased immobility in the FST and TST tests, from day 14 onwards and lasting up to 28 days post-BCG administration. We also observed a significant increase in indoleamine 2, 3-dioxygenase (IDO) activity in the lungs on day-14 post BCG, which is the rate limiting enzyme involved in the synthesis of serotonin from tryptophan and plays major role in cytokine-induced depression. Investigation of acute antidepressant treatment showed that the depressive behavior was resistant to selective serotonin reuptake inhibitors (SSRIs; escitalopram, fluoxetine and citalopram) whereas dual (duloxetine and nomifensine) and triple monoamine reuptake inhibitors (AMR-002) showed an antidepressant effect. Resistance to SSRIs was observed despite achieving high levels of SERT occupancy in BCG-treated mice. In
summary, our results suggest that depression-like behaviors in BCG-treated mice are selectively resistant to acute SSRI treatment.

Disclosures: V.K. Kuchibhotla: A. Employment/Salary (full or part-time):; Bristol Myers Squibb. **A. Rudra:** A. Employment/Salary (full or part-time):; Bristol Myers Squibb. **M.V. Sreedhara:** A. Employment/Salary (full or part-time):; Bristol Myers Squibb. **S. Singh:** A.

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Employment/Salary (full or part-time):; Bristol Myers Squibb.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

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Functional Neurorecovery Pilot Grant, Department of Physical Medicine and Rehabilitation, UAB

Title: Neuroinflammation as a consequence of cardiac ischemia-reperfusion

Authors: *B. JOHNSTON[1], M. E. YOUNG[2], L. L. MCMAHON[3], T. M. DESILVA[1];
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Abstract: While procedural advances have greatly improved survival following heart failure over the past decade, a number of studies have presented evidence for an association between heart failure and cognitive impairment in survivors. Indeed, patients with heart failure have damage to the hippocampus, a brain region critical for normal learning and memory, and reduced volume in the parahippocampal gyrus which projects to the hippocampus. Clearly, further elucidation of the underlying pathophysiology is important for developing therapeutic strategies to prevent cognitive decline in heart failure patients. Ischemic heart disease is the primary cause

of heart failure, which is a chronic failing of the heart to efficiently supply blood to meet the needs of the body. To establish if heart failure results in hippocampal changes in an animal model we used an open and closed-chest acute myocardial ischemia/reperfusion injury. We observed an increase in hippocampal inflammation compared to sham animals within 72 hours of the ischemic event. Furthermore, four months after acute myocardial ischemia/reperfusion deficits in hippocampal learning and memory were observed compared to sham and control animals. We therefore hypothesize that following cardiac ischemia/reperfusion in mice, immediate inflammation ultimately results in cellular damage to the hippocampus, deficits in synaptic function and plasticity at hippocampal synapses, and impairment in hippocampal dependent learning and memory.

Disclosures: B. Johnston: None. **M.E. Young:** None. **L.L. McMahon:** None. **T.M. DeSilva:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 277.19/FFF14

Topic: E.02. Neuroimmunology

Title: REM Selective sleep deprivaction alters intestinal mucosa integrity of wistar rats

Authors: *E. G. IBARRA CORONADO[1], A. M. PANTALEÓN-MARTÍNEZ[1], J. VELÁZQUEZ-MOCTEZUMA[2], A. PÉREZ-TORRES[3], V. RODRIGUEZ-MATA[3]; [1]Inmunologia, Inst. De Investigaciones Biomedicas, UNAM, Mexico DF, Mexico; [2]Dept. de Biología de la Reproducción, Univ. Autonóma Metropolitana - Iztapalapa, México, DF, Mexico; [3]Dept. de Biología Celular y Tisular, Facultad de Medicina, UNAM, México, DF, Mexico

Abstract: Sleep is considered as a fundamental process to maintaining the physiological functions of an organism. It has been shown that sleep deprivation affects the development of and adequate immune response, since it decreased the antibody levels generated against the hepatitis A vaccine. Moreover, specific REM sleep deprivation, produces changes in both number of immune cells and cytokine levels (IL-1, IFNγ, TNFα, IL-6 and IL-17). Addittionally, it has been documented that sleep deprivation promotes the translocation of bacteria from the intestinal mucosa to extra-intestinal sites, compromising the immune system. These findings, show that sleep deprivation alters some of the components related to the immune system, and probably impaired an adequate immune response The aim of our work was to analyze whether REM sleep deprivation alters the mechanisms related to the innate immune response, such as the

integrity of intestinal mucosa, that is considered as the first line of defense against pathogens. Our results, demonstrate that REM sleep deprivation produces an inflammatory infiltrate in the lamina propria of the intestinal villi, which is characterized by an increase in the number of eosinophils and mast cells present in the periglandular area. Concomitantly, there is an increase in expression of IFN-γ and IL-1β. Additionally, epithelium shows an increase in villi detachment, and a decrease in the expression of occludins, which are part of the tight junctions in the intestinal epithelium. Our findings suggests that, the defense mechanisms related to innate immune responses are affected by selective REM sleep deprivation. In this way, translocation of pathogens from the gut, which has been reported in other work, can be mediated by the alteration produced on such molecules involved in maintaining the integrity of the intestinal mucosa, thus it may compromise the ability of the innate immune system to establish an adequate response to infection.

Disclosures: E.G. Ibarra Coronado: None. **A.M. Pantaleón-Martínez:** None. **J. Velázquez-Moctezuma:** None. **A. Pérez-Torres:** None. **V. Rodriguez-Mata:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

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Program#/Poster#: 277.20/FFF15

Topic: E.02. Neuroimmunology

Support: NSF career award (NSF-IOS #0845550)

NIH RO1 (M21068283)

DARPA (W911NF-10-1-0050)

Title: Effects of repeated voluntary or forced exercise on rat brain thermosensitive serotonergic systems

Authors: *M. ARNOLD, B. N. GREENWOOD, J. A. MCARTHUR, P. J. CLARK, M. FLESHNER, C. A. LOWRY;
Integrative Physiol., Univ. of Colorado, Boulder, CO

Abstract: Exposure of rats to elevated ambient temperature activates subpopulations of brainstem serotonergic neurons in the dorsal raphe nucleus (DR), the main source of serotonergic innervation of the forebrain. Thermosensitive subpopulations of serotonergic neurons include those in the "lateral wings" of the DR, also referred to as the dorsal raphe nucleus, ventrolateral

part/ventrolateral periaqueductal gray (DRVL/VLPAG) and those in the interfascicular part of the dorsal raphe nucleus (DRI). DRVL/VLPAG serotonergic neurons are thought to be sympathomotor command neurons involved in inhibition of sympathetic outflow, while DRI serotonergic neurons project to forebrain areas involved in cognitive function and mood, such as the prefrontal cortex and hippocampus, and are thought to be involved in stress resilience. Prior studies have shown that either voluntary or forced wheel running for six weeks has stress resistance effects in rodent models, effects that are dependent on alterations in serotonergic function. It is unclear how voluntary exercise is associated with activation of the DRVL/VLPAG and DRI serotonergic neurons and how this activation is associated with the stress resilient effects of voluntary exercise. Considering the outcomes of the above studies, the objective of the current study was to determine if voluntary or forced wheel running activates DRVL/VLPAG and DRI serotonergic neurons. To investigate this question we conducted two studies. The first study examined expression of the protein product of the immediate-early gene, c-fos, and tryptophan hydroxylase (TPH) using immunohistochemistry in rats exposed to either 1) chronic voluntary exercise for 6 weeks or 2) sedentary control conditions for 6 weeks. The second study included a third treatment group in which rats were exposed to forced exercise for 6 weeks. In Study 1, rats exposed to repeated voluntary exercise had increased c-Fos expression in non-serotonergic neurons within the DRI, relative to sedentary controls. In Study 2, we replicated the findings in Study 1. In addition, rats exposed to repeated forced exercise, but not repeated voluntary exercise, had increased c-Fos expression in serotonergic neurons in the DRI. These results suggest that repeated forced exercise, but not repeated voluntary exercise, activates DRI serotonergic neurons, an effect that may contribute to the recently described stress resilience effects of forced exercise. These results also suggest that the stress resistance effects of chronic voluntary and forced exercise may involve different mechanisms.

Disclosures: M. Arnold: None. **B.N. Greenwood:** None. **J.A. McArthur:** None. **P.J. Clark:** None. **M. Fleshner:** None. **C.A. Lowry:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

Location: Halls B-H

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Program#/Poster#: 277.21/FFF16

Topic: E.02. Neuroimmunology

Support: FRQS

CNS

CIHR

Foundation of stars

Title: Neuronal Interleukin-1β expression initiates inflammation leading to neonatal brain injury induced by combined lipopolysaccharide and hypoxia-ischemia

Authors: *A. SAVARD, D. GRBIC, D. GRIS, G. SEBIRE;
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Abstract: Background: Perinatal hypoxia-ischemia (HI) and infection-inflammation are the principal risk factors of cerebral injuries in newborns. Strikingly, clinical and pathological features of human perinatal brain lesions present variations according to gestational ages. Recently we developed a new preclinical rat model of perinatal induced brain injury at a neurodevelopmental stage equivalent to human term newborn. In this model, lipopolysaccharide (LPS) combined with HI, induced severe damage similar to human brain damage observed in term newborn. Interestigly, the brain lesions were spatially and temporally linked to the expression of IL-1β.
Objective: We hypothesize that neurons constitute the primary source of IL-1β during HI inflammatory challenge. In addition, this neuronal IL-1β triggers cascade of deleterious events leading to exacerbation of brain pathology.
Method: P12 (post-natal day 12) Lewis rat pups were injected with LPS or saline 4h before HI. The right common carotid artery is permanently ligated; 30 min later, pups are exposed to 8% O2 for 1h30.. Brains were studied at different time points for cytokine and chemokine immunoreactivity, polymorphonuclear neutrophil (PMN) infiltration and histological measures of the brain damage. Rats' behaviour assessment was documented after 5 months using Open field, Elevated body swing and Turning in alley tests.
Results: Our results show that IL-1β is strongly expressed early (4 h) after LPS+HI exposition in fronto-parietal neocortical neurones. This neuronal expression is maintained at 24 h and 48 h after LPS+HI exposition with a delayed contribution of glial cells (astrocytes) at 48 h. IL-1β expression then leads to chemokine (MCP-1 and CINC-1) activation and to massive gliosis and neutrophil infiltration starting at 48 h after LPS+HI exposition. LPS+HI-exposed rats showed long-term motor impairments that were prevented by IL-1β blockage. Throughout our experiments we noted only weak increases in the TNF-α expression in microglia and astrocytes.
Conclusion: These results show that neurons are the first cells to express IL-1β and initiate the neuroinflammatory cascades leading to severe brain damage and long term motor impairments in LPS+HI-exposed rats.

Disclosures: A. Savard: None. **D. Grbic:** None. **D. Gris:** None. **G. Sebire:** None.

Poster

277. **Neuroimmunology: Behavioral Effects**

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Program#/Poster#: 277.22/FFF17

Topic: E.02. Neuroimmunology

Support: NIH Grant 5R21NS066130

NIH Grant 1P20GM103643

Title: Effects of morphine on inflammation and the blood-brain barrier in the LP-BM5 AIDS model

Authors: *V. D. MCLANE, L. CAO, C. L. WILLIS;
Col. of Osteo. Med., Univ. of New England, Biddeford, ME

Abstract: Even in the age of highly active antiretroviral therapy, ~20% of human immunodeficiency virus (HIV)-1 infected patients develop cognitive deficits ranging from mild memory loss to dementia. Opioid abuse accelerates cognitive deficit onset and increases central nervous system (CNS) viral load in HIV-1 patients. Opioids such as morphine facilitate the glial inflammatory response to HIV proteins, increasing production of proinflammatory cytokines (tumor necrosis factor-α, interleukin (IL)-1β, regulated on activation, normal T cell expressed and secreted (RANTES, aka CCL5), IL-6, etc.). These cytokines have been shown to reduce blood-brain barrier (BBB) integrity in vivo, which would allow increased access of peripheral virions and infected cells to the CNS, furthering the inflammatory state.
We hypothesized that morphine potentiates BBB breakdown through enhancing CNS inflammatory responses, leading to increased cognitive deficits. To test this, we examined the effects of chronic morphine administration in the LP-BM5-induced murine acquired immunodeficiency syndrome (MAIDS) mode in vivo. 8-week-old male C57Bl/6Ncr mice were injected intraperitoneally with 5x10^4 plaque-forming units of LP-BM5 retroviral mixture. At 7 wks post-infection, morphine (25 mg) or placebo pellets were implanted subcutaneously and replaced 3 days later. Tissues were collected at 8 wks. We then analyzed viral load, cytokine production, and BBB integrity through immunoassays and quantitative real-time (q-rt)PCR. Morphine treatment did not impede the peripheral progression of LP-BM5 infection, as measured by spleen weight, serum IgG and IgM, and tissue viral loads. Spleen cytokine levels did not differ significantly between morphine and placebo-treated LP-BM5 mice. However, CCL5 RNA expression decreased significantly in the frontal lobe of morphine-treated infected mice, suggesting a potential anti-inflammatory role for morphine in the CNS. CNS IL-1β and TNF-α did not show a significant change. Further, immunohistochemistry of the LP-BM5-infected hippocampus revealed increased leak of IgG into the brain parenchyma and loss of claudin-5, a tight junction protein, from the paracellular cleft of brain endothelial cells, indicating

compromised BBB integrity. Morphine treatment did not significantly modify BBB integrity in infected mice. Our work correlates elevated CCL5 with LP-BM5-induced BBB dysfunction. Chronic morphine treatment abolished the CCL5 response, yet did not improve BBB integrity and CNS viral load. There may therefore be a novel pathway for morphine-potentiated blood-brain barrier dysfunction in HIV infection.

Disclosures: V.D. McLane: None. **C.L. Willis:** None. **L. Cao:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 278.01/FFF18

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant RO1HD058638

Title: Plasma membrane progesterone receptors A and B do not complex with dopamine D1 receptor in the arcuate nucleus of the hypothalamus

Authors: *M. MAHAVONGTRAKUL, J. PHAN, K. SINCHAK;
Biol. Sci., California State University, Long Beach, Long Beach, CA

Abstract: Ovariectomized (OVX) rats primed with 2μg of estradiol benzoate (EB) and 26 hours later given 500μg progesterone show maximal sexual receptivity four hours later. EB primes some lordosis facilitative circuits through upregulation of classical progesterone receptors (PR-A & PR-B) necessary for facilitation of lordosis. These PR are classical transcription factors that are localized to the nucleus and cytoplasm. Although PR has been thought to signal primarily in the nucleus, *in vitro* PR expression studies have demonstrated presence of PR-A and PR-B on the plasma membrane (Pedram et al., 2007; Welter et al., 2003). Further, progesterone has been shown to facilitate lordosis within 30 minutes, indicating that a rapid PR signaling mechanism is involved. Similarly, estradiol rapidly activates an inhibitory lordosis circuit through estrogen receptor-α that is trafficked to the plasma membrane (mERα) that complexes with and transactivates metabotropic glutamate receptor-1a (mGluR1a) to activate a multisynaptic circuit originating in the arcuate nucleus of the hypothalamus (ARH). Subsequent progesterone deactivates this circuit to facilitate lordosis, presumably through classical PR signaling. PR are necessary for the estradiol + progesterone facilitation of lordosis, but without dopaminergic neurotransmission via the dopamine D1 receptor (D1), progesterone facilitation of lordosis does not occur (Mani et al., 1994; Mani et al., 1996). However, it is unclear where D1 signaling occurs and whether a direct PR-D1 interaction occurs as seen with mERα and mGluR1a. To

determine whether D1 complexes with PR-A or PR-B on the plasma membrane in the ARH and mediobasal hypothalamus (MBH), OVX rats were treated with oil or 2µg EB and plasma membrane fractions were extracted from block dissections of the ARH and MBH. Although Western blots revealed D1 and PR-A and PR-B were present in the plasma membrane, co-immunoprecipitation demonstrated neither PR-A nor PR-B complexes with D1. These results suggest that although classical PR are found on the plasma membrane in the ARH and MBH, progesterone does not appear to signal through these PR complexed with D1 to facilitate lordosis. Although D1 and PR signaling pathways act through the same lordosis regulatory circuit, D1 may regulate PR interacting with and signaling through Src kinase. Alternatively, progesterone may be acting on a recently discovered group of membrane PR that may be mediated by dopamine signaling.

Disclosures: M. Mahavongtrakul: None. **J. Phan:** None. **K. Sinchak:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

Location: Halls B-H

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Program#/Poster#: 278.02/FFF19

Topic: E.03. Behavioral Neuroendocrinology

Support: RO1HD058638

5R25GM071638

Title: Free estradiol signals through G-protein coupled receptor 30 (GPER) to rapidly facilitate lordosis via deactivation of medial preoptic nucleus-µ-opioid receptors in estradiol primed rats

Authors: *N. P. LONG[1], C. S. SEREY[1], A. WELBORN[2], K. SINCHAK[1];
[1]Biol. Sci., [2]Psychology, California State Univ. Long Beach, Long Beach, CA

Abstract: Facilitation of sexual receptivity (lordosis) can be rapidly induced in a 2 µg estradiol benzoate (EB) primed female rat by a less used paradigm. Instead of progesterone, free 17β-estradiol (E2) can be given 47.5 hours after priming and 30 minutes later lordosis is facilitated. Previously, our research showed that the E2 given 47.5 hours is acting through the G protein coupled estrogen receptor 30 (GPER; aka GPR30) to facilitate lordosis. Antagonism of the opioid receptor-like receptor 1 (ORL-1) blocked the rapid E2 facilitation of lordosis. Estradiol priming initially inhibits lordosis through a multi-synaptic circuit in the arcuate nucleus of the hypothalamus (ARH) that stimulates β-endorphin release that activates and internalizes µ-opioid receptors (MOP) in medial preoptic nucleus (MPN) neurons. Deactivation of MPN MOP by

either 1) subsequent progesterone; 2) exposure to high levels of estradiol for 48 hours, or 3) activation of the ORL-1 (receptor of orphanin-FQ; OFQ) in the ARH facilitates lordosis. We tested the hypothesis that free E2 activation of GPER rapidly facilitates lordosis through deactivation of MPN MOP that is mediated by ORL-1 activation. Ovariectomized Long Evans rats implanted with a third ventricle (3V) cannula were primed with 2 µg EB. DMSO control or E2 was infused into the 3V 47.5 hours later, 30 minutes later rats were tested for sexual receptivity measured by lordosis quotient (LQ). E2 and G1 (GPER agonist) infused rats had a higher LQ compared to the DMSO controls ($P < 0.001$; E2 LQ 88.57 +/- 4.58; G1 LQ 90.00 +/- 7.56; DMSO LQ 6.00 +/- 4.00) which was blocked by pretreatment with G15 (GPER antagonist) or UFP101 (ORL-1 antagonist: $P < 0.05$; G15+E2 LQ 10 +/- 5.16; G15+G1 LQ 16.67 +/- 9.89; UFP101+E2 LQ 5.00 +/- 2.45). Four days later, drug treatments were repeated and brains were processed for MPN MOP immunohistochemistry and MOP activation as measured by MOP immunofluorescence staining intensity (MORi) levels. Infusions of E2 and G1 both deactivated MOP significantly as compared to DMSO controls and pretreatment with G15 and UFP101 ($P < 0.001$; E2 MORi 88.60 +/- 1.33; DMSO MORi 128.31 +/- 2.55; G1 MORi 90.49 +/- 1.27; G15+E2 MORi 127.46 +/- 3.38; G15+G1 MORi 132.09 +/- 1.37; UFP101+E2 MORi 134.81 +/- 1.39). Collectively, these data support our hypothesis that, E2 rapidly signals through GPER to deactivate MPN MOP and facilitate lordosis which is mediated by the activation of ORL-1. Thus, all three steroid priming paradigms signaling mechanisms converge to deactivate ARH β-endorphin neurons that project to the MPN facilitate lordosis.

Disclosures: N.P. Long: None. **C.S. Serey:** None. **A. Welborn:** None. **K. Sinchak:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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Program#/Poster#: 278.03/FFF20

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant R01 MH 50388

Title: Localization of the nucleus accumbens in Japanese quail and European starlings based on hodological, immunohistochemical and functional criteria

Authors: *O. IYILIKCI[1], B. ALWARD[1], A. GILMOUR[1], J. BALTHAZART[2], G. F. BALL[1]; [1]Johns Hopkins Univ., Baltimore, MD; [2]Univ. of Liege, Liege, Belgium

Abstract: In mammals, nucleus accumbens (NAc) is a well-defined ventromedial forebrain structure that plays a critical role in regulating motivation and reward mechanisms. However, the

exact anatomical location of the NAc, its subdivisions and its function in avian species has been a controversial subject. The present study, therefore, aimed to investigate the exact anatomical location of the NAc and its subdivisions based on hodological and histochemical markers. Drawing on these anatomical findings we also aimed to elucidate if the avian NAc plays a role in female appetitive sexual responses similar to its mammalian homologue. Here we examined the hodology of the putative NAc by stereotaxically injecting a biotinylated dextran amine (BDA) as a neuroanatomical tracer in Japanese quail and in European starlings. One week after the injections, subjects' brains were prepared for immunohistochemical labeling of BDA. At low molecular weights (3kDa), BDA retrogradely labels cell bodies that send projections to the injection site. Our results demonstrated afferent inputs to NAc from hippocampus (Hp), ventral tegmental area (VTA), bed nucleus of stria terminalis (BNST), raphe pallidus (Rp), periaqueductal central gray (PAG), lateral preoptic area (lPOA), as well as ventral and dorsal corticoid plate region of mesopallium (MVcp and MDcp). We also examined the distribution of serotonin transporter (SERT) in order to histochemically demarcate NAc and BNST. Our localization of NAc via immunohistochemical markers is in concordance with previous avian studies, which used different immunomarkers. In addition, the afferent hodology of NAc in avian species indicated by the findings of the present study is congruent to its mammalian homologue. Building on these hodological and immunohistochemical findings, we examined the expression of the immediate early gene Zenk in female European starlings that had been exposed to sexually attractive male song. Our results demonstrated an increase in Zenk-immunoreactivity (ir) in response to male song, consistent with a role for the NAc in female sexual motivation. We are also testing Zenk-ir in female Japanese quail in response to conspecific male crows. Overall, the present study explicated the anatomical location of the avian NAc. Additionally, we demonstrated that the hodology and function of avian NAc is congruent with its mammalian homologue.

Disclosures: O. Iyilikci: None. **B. Alward:** None. **A. Gilmour:** None. **J. Balthazart:** None. **G.F. Ball:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIMH R01 50388

CAC is a FRS-FNRS research associate

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University of Liege (special funds) to JB and CAC

Title: Estrogens control female sexual motivation and receptivity in quail

Authors: C. DE BOURNONVILLE[1], G. F. BALL[2], J. BALTHAZART[1], *C. A. CORNIL[1]; [1]GIGA Neurosciences, Univ. Liege, Liege, Belgium; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Estrogens are known to regulate female sexual behavior in quail. Sexual behavior can be divided into different components: appetitive behavior (reflecting motivation) and consummatory behavior (copulatory performance). Recently, it was demonstrated that brain-derived estrogens acutely control male sexual motivation but not performance. In females, repeated peripheral administrations of tamoxifen (an estrogen receptor antagonist) decreases receptivity in females. Whether this effect on receptivity reflects estrogen's action on sexual motivation or performance is not known. To study this question, we designed sexual motivation tests for females in which they could either approach a tethered male in an empty arena or choose to approach either a male or a female that were similarly tethered. Experimental females were treated with tamoxifen (2mg/animal/day) or the vehicle for 15 consecutive days. Treatment was then stopped for 18 days to test for a possible recovery. Females were repeatedly tested for sexual motivation and sexual receptivity throughout the experiment. Female receptivity and motivation to approach a male were strongly decreased by the tamoxifen treatment. During the 18-day recovery period, tamoxifen-treated females significantly increased their receptivity but did not completely recover to their basal level of receptivity. In conclusion, these results suggest that the deficit in receptivity induced by estrogen blockade likely results from a reduction in sexual motivation in female quail. Future studies should investigate the source of the estrogens mediating these motivational effects. Indeed, although female ovaries produce large amounts of estrogens, the female brain also contains aromatase, the rate-limiting enzyme for estrogen synthesis. Brain aromatase in females is controlled by estrogens (inhibited by chronic tamoxifen treatment) and can be rapidly regulated by environmental stimuli. It is thus conceivable that, as in males, some of the neuro-active estrogens in females are produced locally.

Disclosures: C. de Bournonville: None. **G.F. Ball:** None. **J. Balthazart:** None. **C.A. Cornil:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH/NINDS RO1 35467

Title: Differential effects of global versus local testosterone on song behavior and its underlying neural substrate

Authors: *B. A. ALWARD[1], J. BALTHAZART[2], G. F. BALL[1];
[1]Psychological and Brain Sci., The Johns Hopkins Univ., Baltimore, MD; [2]GIGA Neurosciences, Univ. de Liege, Liege, Belgium

Abstract: Testosterone (T) has multiple effects on sexually-motivated song in male songbirds, regulating both the motivation to sing as well as its quality. However, the brain region(s) where T acts to regulate these different aspects of song behavior have not been definitively characterized. One candidate site where T might exert its influence on song motivation is the medial preoptic nucleus (POM). POM lesions block male-typical sexual behaviors and the action of T in the POM is sufficient to activate male sexual motivation and performance in birds and mammals. We hypothesized that song, at least in part, is regulated by T action in the POM. We tested this by implanting T into the POM of castrated male canaries (Border strain) and assessing song rate and acoustic features as well as stereotypy, a measure of song quality. We also presented a female to males to assess the activation of sexual behaviors in her presence. We demonstrate that T in the POM was sufficient to induce song rate to levels similar to those in birds with globally circulating T, but took longer to exhibit a significant difference from baseline levels as compared to castrated birds with blank implants (about 9 days versus 3). On the other hand, T in the POM was unable to induce song stereotypy to levels of birds with global T. Castrated birds treated with T peripherally had larger volumes of song nuclei as compared to castrates with blank implants but strikingly canaries receiving T in the POM had song control nuclei volumes as large as birds receiving global T, suggesting activity-induced neuroplasticity. We also show that T in the POM is sufficient to activate copulation in canaries but not the number of calls or peri-copulatory songs made in her presence. These results suggest that while T in the POM is sufficient for activating song rate and copulation, T must act elsewhere to induce normal song latency, stereotypy, as well as vocalizations made in the presence of a female. These results support the conserved role of T actions in the POM in regulating male sexual behaviors specifically and that T acts at multiple brain loci to regulate specific aspects of singing behavior in songbirds.
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Disclosures: B.A. Alward: None. **G.F. Ball:** None. **J. Balthazart:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF Grant IOS-0742833

Title: Androgen receptor protein is increased in the hypothalamus of male compared to female green anole lizards during the breeding season

Authors: *H. KERVER, J. WADE;
Neurosci., Michigan State Univ., East Lansing, MI

Abstract: Green anole lizards are seasonal breeders, with an annual rise testosterone (T) regulating male sexual behavior. T has dramatic effects on masculine behavioral, morphological, and biochemical functions during the breeding season (BS), but these effects are not mirrored in the non-breeding season (NBS) and are limited in females. Data suggest that T acts at androgen receptors (AR). Therefore, we hypothesized that increased AR protein expression in the hypothalamus in the BS facilitates the seasonal responsiveness to T in males. We also hypothesized that two AR coactivators, steroid receptor coactivator-1 (SRC-1) and CREB binding protein (CBP), are involved. Here we performed Western blot analyses on hypothalamic samples dissected from adult males and females from both the BS and NBS. Two experiments were completed, the first on gonadally intact animals and the second on gonadectomized animals that received either a blank or T-filled pellet. No significant effects of sex or season were detected for any of the three proteins in the intact animals (all $F \leq 1.47$, $p \geq 0.239$). Manipulated animals in the second study also exhibited no main effects of sex, season, or treatment for AR ($F \leq 2.23$, $p \geq 0.333$), but there was a significant sex x season interaction ($F=14.10$, $p<0.001$). A two-way ANOVA for sex and season confirmed this interaction ($F=15.20$, $p \leq 0.001$), and within the BS, AR was increased in the hypothalamus of males compared to females ($t=4.11$, $p<0.001$). There were no differences in AR expression between sexes in the NBS ($t=1.79$, $p=0.086$) or across seasons in males ($t=1.70$, $p=0.100$). Among females, more AR was expressed in the NBS than BS ($t=3.74$, $p=0.001$). Treated animals showed no effects on CBP ($F \leq 0.90$, $p \geq 0.347$) or SRC-1 expression ($F \leq 1.19$, $p \geq 0.282$). Differences between the two experiments may be due to increased sample size and/or removal of gonadal influences in the second study. Collectively, the results are consistent with the idea that relative levels of AR expression may facilitate sex differences in hypothalamic structure and function. However, it does not appear that AR, SRC-1 or CBP in this general region of the brain regulate seasonal differences in responsiveness to T.

Disclosures: H. Kerver: None. **J. Wade:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC to JGP

Undergraduate summer NSERC scholarship to JGG

Title: Effects of chronic RU486 on the sensitization of sexual behaviors by repeated estradiol benzoate in the ovariectomized Long-Evans rat

Authors: *S. JONES, J. GARDNER GREGORY, J. G. PFAUS;
CSBN/Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Sexual behaviors in the ovariectomized (OVX) rat are partially restored by an acute injection of estradiol benzoate (EB), and fully restored by a subsequent injection of progesterone (P). Repeated injections of 5µg or 10µg EB, but not 2µg EB, administered alone every 4 days to sexually-experienced OVX rats results in a the sensitization of sexual behaviors, since successively greater sexually appetitive behaviors (hops, darts, & solicitations) and lordosis quotients (LQ) are observed. Previous research has suggested that P receptors are not involved in the expression of sexual behavior by estradiol alone (Blaustein et al., 1987). Here we tested whether chronic injections of the P receptor antagonist, RU486, would alter the development of behavioral sensitization by repeated injections of EB. Females were treated with EB (5 or 10µg), and 5mg RU486 dissolved in 0.4mL vehicle (VEH; 80% sesame oil, 15% benzyl benzoate, 5% benzyl alcohol) 48 and 4 hours prior to each of 7 tests, respectively, occurring at 4-day intervals in unilevel 4-hole pacing chambers. Control animals were treated with 2, 5, or 10µg EB+VEH. As expected, sensitization did not occur in females treated with 2µg EB+VEH. LQ sensitized across tests in all other groups, however RU486 attenuated LQ on the two final test days. Appetitive behaviors sensitized in the RU486 groups, however this sensitization was also attenuated on the two final test days. Interestingly, appetitive sexual behaviors did not sensitize in females treated with VEH. In accordance with Pleim et al., (1990), RU486 given in combination with 5µg, or 10µg EB, facilitates sexual behavior by inducing more appetitive sexual behaviors, and higher LQ compared to VEH. Despite the initial facilitation, however, blocking P receptors by chronic administration of RU486 appears to inhibit, or disrupt the maintenance of, the sensitization of sexual behaviors by repeated administration of EB.

Disclosures: S. Jones: None. **J. Gardner Gregory:** None. **J.G. Pfaus:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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CONACYT 167101(RP)

CONACYT 152872 (WP)

Title: Effects of progestins in the modulation of sexual receptivity in female mice

Authors: L. E. GRIJALVA[1], O. GONZÁLEZ-FLORES[2], F. J. CAMACHO[1], *N. F. DIAZ[3], S. K. MANI[4], R. G. PAREDES[1], W. PORTILLO[1];
[1]Inst. de Neurobiología UNAM, Queretaro, Mexico; [2]Ctr. de Reproducción Animal de la Univ. Autónoma de Tlaxcala-CINVESTAV, Tlaxcala, Mexico; [3]Cell Biol., Inst. Nacional de Perinatologia, Mexico D.F., Mexico; [4]Baylor Col. of Med., Houston, TX

Abstract: Progesterone (P) can be rapidly metabolized in the central nervous system to the ring A reduced metabolites progestins. These, steroids have low affinity for the P receptor (PR), but induce female sexual receptivity with a higher potency than P in estrogen primed rats. In addition, the receptivity induced by P is followed by a period in which female do not respond behaviorally to a second administration of P (sequential inhibition). The aim of the present study was to evaluate the role of several ring A reduced progestins to induce sequential inhibition in estrogen primed female mice. Female mice that were treated with estradiol benzoate (EB) plus P which 24 hours later received a second dose of P, were not sexually receptive. Female mice treated with EB plus 5b Pregnanedione (5b DHP), 5a3b or 5b3a Pregnanolone (5a3b PGL and 5b3a PGL respectively) a second P dose (24 hours later), induced high levels of receptivity. These observations indicate that progestins with low affinity for the PR do not induce sequential inhibition. We also evaluated the effect of ring A reduced progestins in mutant mice, which are deficient in PR (PRKO) and compared to their wild type (WT) littermates. WT females treated with EB plus P administration showed high levels of receptivity compared to the PRKO. Administration of EB plus 5b3a or 5a3b PGL facilitated high levels of receptivity in WT and

PRKO animals. Furthermore, WT females treated with EB plus 5b3a or 5a3b PGL that received P 24 h later showed high levels of receptivity. The same treatment failed to induce receptive behavior in PRKO females. Taken together, these data suggest that progestins do not induce sequential inhibition. They also show that 5b3a or 5a3b PGL can induce sexual receptivity by an independent mechanism of nuclear PR.

Disclosures: L.E. Grijalva: None. **O. González-Flores:** None. **F.J. Camacho:** None. **N.F. Diaz:** None. **S.K. Mani:** None. **R.G. Paredes:** None. **W. Portillo:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC to JGP

Title: Ovarian steroids modulate dopamine receptor binding in the medial preoptic area of female rats

Authors: *M. GRAHAM, D. HUSSAIN, W. G. BRAKE, J. G. PFAUS;
CSBN-Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Dopamine (DA) transmission in the medial preoptic area (mPOA) plays a critical role in the control of appetitive sexual behavior in the female rat. We have shown previously that a DA D1R-mediated excitatory state appears to occur in females primed with estradiol benzoate (EB) and progesterone (P), whereas a DA D2R-mediated inhibitory state appears to occur in EB-alone females. We have also found evidence of DA receptor (DAR) modulation by hormonal administration, through examination of protein levels using the Western blotting technique. These data indicate that females treated with EB+Oil show reduced DA D1R in the mPOA, while adding progesterone (EB+P) increases these receptor levels. A similar effect was found in the prelimbic cortex (PLC), but no differences were found in the caudate putamen (CP), control areas examined due to their role in female sexual behaviour and presence of high levels of DA. To better understand the changes that occur to DAR following hormone administration, the present experiment used in vitro quantitative receptor autoradiography to quantify the amount of DA D1R binding levels in the mPOA, CP, and PLC. Ovariectomized rats were randomly assigned to one of three steroid treatment groups: EB+P (10 μg and 500 μg, respectively), EB+Oil, Oil+Oil. Hormone injections were administered 48h and 4h prior to sacrifice, and the density of binding sites in the CP, PLC, and mPOA were compared following incubation periods

of 4, 6, and 12 weeks, respectively. Similar to the results of the Western blotting, hormonal administration alters DA D1R binding levels in the mPOA, as females treated with EB+Oil show decreased binding levels, and those in the EB+P group show increased binding levels. Binding levels did not differ in the other two areas examined. These data confirm the DA receptor protein changes observed in our previous studies, and provide further evidence that EB+Oil females may have a low DA D1R/D2R ratio, and EB+P females may have a high DA D1R/D2R ratio.

Disclosures: M. Graham: None. **D. Hussain:** None. **W.G. Brake:** None. **J.G. Pfaus:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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Topic: E.03. Behavioral Neuroendocrinology

Support: Gilman Grant: Carleton Faculty Development Endowment

Title: Absence of pubertal ovarian hormone exposure induces higher activity, but not receptivity, in hormone-primed adult female rats

Authors: *S. H. MEERTS, M. E. FARRY-THORN, L. K. DAIRAGHI, W. KAY, N. MASON, R. S. SCHAIRER;
Carleton Col., Northfield, MN

Abstract: The female phenotype has long been thought to be the default developmental pathway, occurring in the absence of gonadal hormone exposure, but recent evidence has demonstrated prepubertal organizational effects of estradiol in females. The role of estradiol in organizing the female brain during puberty is not well understood. We hypothesized that without pubertal exposure to ovarian hormones, female rats would show more feminine behavior in adulthood when primed with estradiol benzoate (EB) and progesterone (P) due to a heightened sensitivity to EB/P. Here we tested whether pubertal ovarian hormones affect the display of lordosis and activity in adulthood following hormone treatments known to induce either full or partial receptivity. Female Long-Evans rats were ovariectomized before puberty (No-O@P) or after puberty (O@P). Four weeks after ovariectomy rats received two cycles of EB/P in the two weeks before experimental procedures began to facilitate vaginal opening in No-O@P rats. Receptivity was assessed with a 10-stimulation lordosis quotient (LQ) test followed immediately by a 10-min open field activity test. Rats were tested for receptivity and activity after full hormone priming (10 ug EB 48h and 1 mg P 4h prior to tests) and one week later after administration of subprime hormone treatment (2 ug EB 48h and 500 mg P 4h prior to tests).

O@P and No-O@P rats did not differ in terms of receptivity under either hormone-priming regimen, but with subprime hormone treatment No-O@P rats took significantly longer to receive 10 stimulations in the LQ test and exhibited significantly more arena crossings in the open field test than O@P rats. The elevated activity in No-O@P rats could either have been due to the absence of pubertal hormone exposure alone or in combination with the neuroendocrine cascade induced by the preceding mating. Therefore, the rats received two additional open field tests. First, rats received full hormone priming prior to mating and then activity was assessed after rats received 15 intromissions. One week later, rats were again fully EB/P-primed and activity assessed with no preceding mating. No-O@P rats only showed significantly elevated activity levels relative to O@P rats when tested immediately after mating, suggesting that some aspect of mating triggers elevated activity in No-O@P rats. Thus, the presence or absence of ovarian hormones during puberty affects activity, but not receptivity, indicating that the female rat brain is organized to some degree by pubertal ovarian hormones. Additional research is necessary to pinpoint the role of ovarian hormones in pubertal organization of the female rat brain.

Disclosures: S.H. Meerts: None. **M.E. Farry-Thorn:** None. **L.K. Dairaghi:** None. **W. Kay:** None. **N. Mason:** None. **R.S. Schairer:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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Program#/Poster#: 278.11/GGG2

Topic: E.03. Behavioral Neuroendocrinology

Support: CIHR

Title: Vasopressin and dopamine neurons are activated in female rats that display conditioned mate-guarding behavior

Authors: *A. HOLLEY, S. BELLEVUE, S. ROORDA JR, D. VOSBERG, J. G. PFAUS; Concordia Univ., Montreal, QC, Canada

Abstract: Rats are often described as having a polygamous mating strategy, yet through simple Pavlonian conditioning paradigms, where neutral odors are paired with sexual reward states, rats can develop conditioned sexual partner preferences, where they prefer to solicit, copulate, and mate with scented familiar partners over unscented unfamiliar ones. We have previously shown that ovariectomized female rats primed fully with estradiol and progesterone do not need odors if they are paired with the same male for their first 10 paced sexual experiences: They are able to recognize their partner male based on primary cues alone and will display rudiments of mate-

guarding behavior when placed in a situation where defending their mate is required. In the present study we used double-immunocytochemistry to examine the induction of Fos protein within vasopressin neurons of the PVN and SON, or within the dopamine neurons of the ZI, VTA, and SN of females that display conditioned mate guarding in the presence of their familiar male and another female in an open field, relative to females that copulated with a random male and another female. Conditioned rats showed significant activation of vasopressin neurons in both regions relative to unconditioned, non-partner rats. Conditioned rats also showed significant activation of dopamine neurons in the ZI and VTA compared to unconditioned rats. These findings indicate that female rats are not hard-wired to be polygamous, and that early experience with sexual reward that is conditioned with the same male induces conditioned mate-guarding behavior in the presence of that male when a novel female is present as a competitor. This behavior is accompanied by significant induction of Fos within vasopressin and dopamine neurons relative to the near absence of vasopressin and dopamine activation in non-partner females who copulate but do not display conditioned mate-guarding behavior.

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Poster

278. **Hormonal and Neural Control of Sexual Behavior**

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Topic: E.03. Behavioral Neuroendocrinology

Support: CONACYT 167101

PAPPIT IN200512

PAPPIT IB20011

Title: Gene expression in the mPOA of copulating and sexually sluggish male rats

Authors: A. GONZÁLEZ-GALLARDO, *W. PORTILLO, C. GARCÍA-UGALDE, R. G. PAREDES, A. ANTARAMIAN;
NEUROBIOLOGIA CONDUCTUAL Y COGNITIVA, INSTITUTO DE NEUROBIOLOGIA, QUERETARO, Mexico

Abstract: Sexually sluggish males have been described in several species including rats. These animals take more than 30 min to ejaculate or they ejaculate inconsistently despite being tested

repeatedly with sexually receptive females. Different brain areas and hormones play an important role in the control of male sexual behavior. The aim of the present study was to determine the expression of the vitamin D receptor (VDR), the androgen receptor (AR), the estrogen receptor alpha (ERa), the progesterone receptor (PR), and the aromatase enzyme (P450) genes in the medial preoptic area (mPOA), the amygdala (AMG) and the olfactory bulb (OB) of copulating (C) and sexually sluggish (SS) male rats. Subjects were tested with sexually receptive females in 3-4 tests of sexual behavior. Those males that ejaculated within 30 min in all tests were included in the C group and those males that ejaculated in one or none of the 4 tests were included in the SS group. One week after the last test of sexual behavior, males were sacrificed by decapitation to obtain the different brain regions. RNA was isolated and cDNA synthesized from the areas described, with cortex (CTX) included as a control group. Samples were dissected from 8 C and 8 SS males. Expression of the VDR, P450, AR, ERa and PR genes was determined by qPCR. PCR efficiency of all genes was determined using serial dilutions of mPOA cDNA. The geometric mean of the Ct from two housekeeping genes, cyclophilin A and tyrosine 3-monooxygenase-tryptophan activation protein zeta, was used to determine relative gene expression using the 2-double dCt method. Gene identity was confirmed by sequencing. In the mPOA, P450 mRNA increased 1.35 fold in SS males versus C males. AR mRNA also increased 3.45 fold and VDR showed 79.55 fold increase in the mPOA of SS males; only ERa and PR exhibited no change in this area. P450 mRNA was reduced 0.399 fold in the AMG. VDR and AR were not detectable in the OB, nor was AR in the AMG. Neither VDR nor P450 were detectable in the CTX. In the OB the only mRNA expressed at different levels in SS males was ERa (0.68). In conclusion, expression of several genes involved in hormone responsiveness is altered in the mPOA of SS males that could be associated with the low levels of sexual behavior.

Disclosures: A. González-Gallardo: None. **W. Portillo:** None. **R.G. Paredes:** None. **C. García-Ugalde:** None. **A. Antaramian:** None.

Poster

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Title: Repeated sexual behavior and neurogenesis in the hippocampus of the female rat

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Abstract: The ability of female rats to control the rate of sexual interactions (pacing) has important physiological and behavioral consequences. We have previously shown that the first paced mating encounter increases the number of newborn cells in the accessory olfactory bulb (AOB) in the female rat. Furthermore, when the females are allowed to paced the sexual interaction once weekly for 4 consecutive weeks this effect is potentiated and a higher number of new neurons is observed in the accessory and main olfactory bulbs. In the present study we evaluated if the repetition of the stimulus (paced mating) could increase the survival of newborn neurons, in the dentate gyrus (DG), generated during the first session of paced sexual contact. Sexually-naive female rats were bilaterally ovariectomized, hormonally supplemented and randomly assigned to one of four groups: 1) without sexual contact, 2) one session of paced mating during 1h, 3) four sessions of paced mating of 1h each and 4) four sessions of non-paced mating during 1h. Results showed an increase in the number of newborn cells (BrdU immunoreactive) in the ventral, dorsal and total (ventral + dorsal) DG of the repeated paced mating group in comparison to the control group. These results indicate that repeated paced mating promotes newborn neurons survival in DG of the hippocampus, which is consistent with our previous results in the olfactory bulb. We thank Francisco Camacho for his technical assistance.

Disclosures: M.G. Ortiz: None. **R.G. Paredes:** None. **M.C. Sanchez:** None. **D.M. Arzate:** None. **W. Portillo:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 278.14/GGG5

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant MH040826

Title: Carbon monoxide neurotransmission in the MPOA plays a role in male rat sexual and anxiety-like behaviors

Authors: *C. L. ROBISON[1], J. A. MCHENRY[2], E. M. HULL[2];
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Abstract: Carbon monoxide (CO) has been implicated as a gaseous neurotransmitter involved in functions as diverse as synaptic plasticity, vasorelaxation, and neuroendocrine regulation. The primary source of CO in the brain is the enzyme heme oxygenase 2 (HO-2). Given its similarities with nitric oxide (NO) neurotransmission, we hypothesized that CO plays a role similar to that of NO in the medial preoptic area (MPOA) of male rats. We observed an increase in HO-2 immunoreactivity in the MPOA after repeated, but not a single, sexual experience, similar to the increase in the NO-producing enzyme NOS. Administration of the HO-2 antagonist, tin mesoporphyrin IX (SnMP) to the MPOA prior to each of seven non-contact exposure to a sexually receptive female impaired sexual behavior on subsequent copulatory behaviors 1-2 days after the last exposure compared to vehicle controls. SnMP administration also elicited unusual behaviors such as excessive climbing during the non-contact exposure but did not appear to affect locomotion or coordination. Animals administered SnMP to the MPOA ten minutes before a copulatory encounter did not express altered copulatory or locomotor behavior compared to controls, but displayed significantly longer latency to reach and intromit with a receptive female in an X-maze task, indicating that CO may facilitate motivational or learning aspects of sexual function, but unlike NO does not centrally affect copulatory performance. Conversely, animals administered SnMP to the MPOA ten minutes before an elevated plus-maze task spent significantly less time in open arms and more time in closed arms compared to controls while expressing normal locomotor activity. Thus, the blockade of CO neurotransmission in the MPOA inhibits learning and motivational aspects of copulatory function while increasing the expression of anxiety-like behaviors in the male rat.

Disclosures: C.L. Robison: None. **J.A. McHenry:** None. **E.M. Hull:** None.

Poster

278. **Hormonal and Neural Control of Sexual Behavior**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 278.15/GGG6

Topic: F.03. Motivation and Emotion

Title: Pontine control of ejaculation and female orgasm

Authors: *G. HOLSTEGE[1], A. T. M. WILLEMSEN[2], H. K. HUYNH[3], T. A. LOVICK[4];
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Netherlands; [3]Ctr. for Uroneurology, Haren, Netherlands; [4]Sch. of Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom

Abstract: Ejaculation is a key event and end point in male sexual activity. At the climax of coitus ejaculation propels seminal fluid containing sperm through the urethra and into the female vagina, the biological objective being to fertilize a female ovum. This event is effected by a highly synchronized pattern of rhythmic contractions of smooth and striated muscles within the pelvic region.
Although ejaculation is a purely male event, a homologue exists in females. During female orgasm, the vaginal smooth muscle and the striated bulbospongiosus muscle produce rhythmic contractions in the distal part of the vagina to draw semen into the vagina, making it easier for sperm to approach and fertilize the egg.
The physiological component of ejaculation shows parallels with micturition since both are essentially voiding activities. Both depend on supraspinal influences to orchestrate the characteristic pattern of activity in the pelvic organs. Unlike micturition, little is known about the supraspinal pathways involved in ejaculation and female orgasm.
Ejaculation in men and orgasm in women was induced by manual stimulation of the penis or clitoris by the participants' partners. Positron emission tomography (PET) with correction for head movements was used to capture the pattern of brain activation at the time of sexual climax.
Ejaculation in men and orgasm in women resulted in activation in a localized region within the dorsolateral pontine tegmentum on the left side and in another region in the ventrolateral pontine tegmentum on the right side. The dorsolateral pontine area was also active in women who attempted but failed to have an orgasm and in women who faked orgasm. The ventrolateral pontine area was only activated during ejaculation and physical orgasm in women.
Activation of a localized region on the left side in the dorsolateral pontine tegmentum, which we termed the pelvic organ stimulating center (POSC) occurs during ejaculation and physical orgasm in women. This same region, but on the right side, has previously been shown to be activated during micturition. The POSC, via projections to the sacral parasympathetic motoneurons, controls pelvic organs involved in voiding functions. In contrast, the ventrolateral pontine area, which we term pelvic floor stimulating center (PFSC), produces the pelvic floor contractions during ejaculation and physical orgasm in women via direct projections to pelvic floor motoneurons.

Disclosures: G. Holstege: None. **A.T.M. Willemsen:** None. **T.A. Lovick:** None. **H.K. Huynh:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.01/GGG7

Topic: E.04. Autonomic Regulation

Support: NIH Grant NS056314

Title: A role of Lis1 in adult mice

Authors: *X. GAO, D. SMITH;
Univ. of South Carolina, Columbia, SC

Abstract: *Lis1* haploinsufficiency in humans results in a "smooth brain" phenotype called lissencephaly, and also causes severe cognitive and motor impairments and epilepsy. Various mouse models have been used to examine the role of Lis1 during brain development, and it is clear that Lis1 regulates a microtubule motor, cytoplasmic dynein. Intriguingly, Lis1 expression remains high in adult brains indicating that it plays a role in mature systems. Indeed, our group found that Lis1 and several related proteins regulate dynein-dependent axon transport in cultured adult rat sensory neurons. I am interested in examining Lis1 function in the adult mouse, and in order to bypass the embryonic impact of Lis1 loss I have utilized a tamoxifen inducible Cre-mediated recombination system to knock out Lis1 in adult mice (8 weeks). I chose to cross floxed Lis1 mice with mice in which Cre expression is driven by an actin promoter. I find that loss of Lis1 in adult mice causes severe malaise and ultimately death. The timing of the lethality is dependent on the tamoxifen dose. These phenotypes are not observed in any of a variety of control animals. Thus, Lis1 plays a crucial role in an adult mammalian system. I examined the pattern of Cre activation in experimental animals, and found that Cre is activated in several tissues including brain stem, heart and skeletal muscle. Lis1 is normally expressed at substantially higher levels in brain than in heart, and no Lis1 was detected in skeletal muscle. Cre activation led to reduced Lis1 level protein expression in brainstem and heart. We hypothesize that the sudden death of Lis1 conditional knock out mice may be related to the cardiorespiratory control network in the brainstem, but could conceivably reflect a hitherto unforeseen role for Lis1 in cardiomyoctes.
R01 NS056314
National Institutes of Health(NINDS)

Disclosures: X. Gao: None. **D. Smith:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.02/GGG8

Topic: E.04. Autonomic Regulation

Support: NSC 100-2321-B-075B-002

Title: Prazosin reverses acute apoptosis in a rat model of enterovirus 71 encephalitis

Authors: *C.-J. TSENG[1], W.-H. LU[2], K.-S. HSIEH[2], L.-P. GER[1];
[1]Veterans Gen Hosp-Kaohsiung, Kaohsiung, Taiwan; [2]Dept. of Pediatrics, Kaohsiung Veterans Gen. Hosp., Kaohsiung, Taiwan

Abstract: Children infected with enterovirus 71 encephalitis progressing abruptly to left ventricle dysfunction and neurogenic pulmonary edema die suddenly within hours. In our previous study, we identified a rat model with nucleus tractus solitarii lesion causing immediate excessive catecholamine release to mimic fulminant enterovirus 71 infections. In this study, we investigated the alpha-adrenergic blockers on survival in this rat model. 270-360g male Sprague-Dawley rats were divided into two groups. Group 1 was a control group that received microinjections of 6-hydroxydopamine into the nucleus tractus solitarii bilaterally. Group 2 was an intervention group with α-adrenergic blocker (prazosin). We found that prazosin can prevent cardiac hypercontracture and preserve cardiac output. It also reverses the pathological changes of contraction band necrosis, increased expressions of brain natriuretic peptide and troponin T in the heart and neutrophil infiltration in the lungs, leading to prevention of hyaline membrane formation and pulmonary hemorrhagic edema, increasing survival time. The time-dependently increased and strong expression of connexin 43, caspase3 and the apoptotic cells induced by the 6-hydroxydopamine lesion were decreased after prazosin treatment. Connexin 43 is involved in a novel apoptotic mechanism for speeding apoptosis within hours in the rat model. For translational research on fatal enterovirus 71 encephalitis, early prazosin treatment may prevent acute death from tissue apoptosis.

Disclosures: C. Tseng: None. **W. Lu:** None. **K. Hsieh:** None. **L. Ger:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.03/GGG9

Topic: E.04. Autonomic Regulation

Support: NSC102-2320-B-075B-002

Title: Wnt signaling activation in nucleus tractus solitarii modulates insulin receptor substrate-mediated central blood pressure regulation

Authors: *W.-H. CHENG[1,2], C.-J. TSENG[1,2];
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Abstract:
The cluster of pathologies known as metabolic risk factors, including high blood pressure, high fasting blood sugar, and insulin resistance, has become one of the most serious warnings of human health. The nucleus tractus solitarius (NTS), located at the dorsal part of the brainstem, was discovered to be an important sympathetic nervous system integral center in the central nervous system (CNS). Interestingly, our previous studies also mentioned that insulin plays a role in central cardiovascular regulation. Moreover, insulin signaling is defective in the NTS of hypertensive spontaneously hypertensive rats (SHRs) and fructose-fed rats. The Wnt signaling is involved in many physiological and pathophysiological activities. In addition, recent clinical and in vitro studies reveal that aberrant Wnt signaling is thought to play an important role in the onset of diabetes. Nevertheless, the relationship between Wnt signaling and insulin pathway and related modulation of blood pressure in the CNS has not been established. In light of these observations, we postulated that Wnt signaling pathway may improve neuronal insulin resistance and that neuronal insulin resistance is a key mechanism involved in triggering hypertension. Intracerebroventricular administration of Wnt3a produced depressor in both SHRs and fructose-fed rats. Pretreatment with a low density lipoprotein receptor-related protein (LRP) antagonist, Dickkopf-1 (DKK-1) significantly attenuated the depressor effect and nitric oxide production evoked by Wnt3a. Additionally, immunoblotting and pharmacological studies further showed inhibition of LRP6 activity by DKK-1 significantly abolished Wnt3a-induced glycogen synthase kinase 3beta (GSK-3beta)^{S9}, extracellular signal-regulated kinases 1/2 (ERK1/2)$^{T202/Y204}$, ribosomal protein S6 kinase (RSK)$^{T359/S363}$, Akt^{S473} and increased insulin receptor substrate-1 (IRS1)^{S332} phosphorylation. GSK-3beta was also found to direct bind to IRS1 and induced Ser332 phosphorylation in the NTS. Likewise, the addition of GSK-3beta inhibitor (TWS119) into the brain decreased blood pressure of SHRs and fructose-fed rats through enhancing IRS1 activity. Taken together, these results suggest that the Wnt-LRP6-GSK3beta signaling may as a key regulator of insulin pathway and a potential therapeutic target for essential hypertension.

Disclosures: W. Cheng: None. **C. Tseng:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.04/GGG10

Topic: E.04. Autonomic Regulation

Support: FAPESP2011/23513-8

Title: Genic expression of oxytocin in the paraventricular nucleus is altered by paroxetine in subchronic aortic regurgitation rats

Authors: *J. I. GOBBI[1], A. M. OMOTO[1], L. N. MORAES[2], M. G. ROSCANI[3], L. S. MATSUBARA[3], B. B. MATSUBARA[3];
[1]Dept. of Physiol., [2]Dept. of Morphology, UNESP, Inst. of Biosci., Botucatu, Brazil; [3]Dept. of Intrnl. Med., UNESP, Med. Sch., Botucatu, Brazil

Abstract: Aortic regurgitation (AR) leads to eccentric hypertrophy and eventually left ventricular dysfunction. Rheumatic heart disease is a leading cause of AR in developing countries, and its incidence is increasing in developed countries as a consequence of senile degeneration. Depression and heart disease are correlated states. Alterations in the metabolism of serotonin (5-HT) are particularly implicated. Selective 5-HT reuptake inhibitor (SSRI) are widely used as antidepressants. Treatments with 5-HT agonists or 5-HT releasers facilitate oxytocin (OT) and vasopressin (AVP) secretion in the plasma. OT regulates cardiac function and hydromineral balance. The hypothalamic-neurohypophyseal system is responsible for OT and AVP secretion. Thus, the present study was designed to test the hypothesis that paroxetine (a SSRI) treatment changes paraventricular hypothalamic (PVN) OT and AVP expression in rats with subchronic aortic regurgitation. Male Wistar rats (260 to 280 g) had sham or AR surgeries induced by retrograde puncture of the aortic valve leaflets, procedures were aproved by animal ethical commitee (386-CEUA/SBCAL). Presence of AR was confirmed by echocardiography (Eco) exams. Rats were randomly divided into 4 groups: AR+parox, AR+control, sham+parox and sham+control. Eco exams were conducted for collecting morphological variables at week 4 following AR surgeries. The groups started to be treated with parox (10 mg/kg 3 times in a week) or control subcutaneous injections till week 8, when Eco exams were conducted again in order to verify systolic function. Rats were deeply anesthetized. Fresh brains were frozen and sliced to isolate the PVN. Relative OT and AVP mRNA expression in the PVN was quantified by real-time PCR. As result fractional shortening was preserved in AR+parox, compared to AR+control (47.1 ± 1.5% vs 33.0 ± 4.0%, respectively). AR+parox had an increased in the OT expression (0.61 ± 0.16 vs 0.42 ± 0.08 relative quantification). AVP was not changed. The results suggests that increasing central 5-HT levels, determined by the antidepressant, may change oxytocin PVN expression which produces improvements to cardiovascular system exposed to volume overload.

Disclosures: J.I. Gobbi: None. **A.M. Omoto:** None. **L.N. Moraes:** None. **M.G. Roscani:** None. **L.S. Matsubara:** None. **B.B. Matsubara:** None.

Poster

279. **Cardiovascular Regulation I**

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Program#/Poster#: 279.05/GGG11

Topic: E.04. Autonomic Regulation

Support: NIH Grant HL076803

NIH Grant HL083810

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Title: Diet-induced obesity is associated with angiotensin-dependent microglial activation, astrogliosis and inflammation in the hypothalamus

Authors: *A. D. DE KLOET[1], D. NGUYEN[2], D. J. PIOQUINTO[1], L. WANG[2], E. G. KRAUSE[2], C. SUMNERS[1];
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[2]Pharmacodynamics, Univ. of Florida, Gainesville, FL

Abstract: Obesity and co-morbid hypertension are epidemic health concerns and major risk factors for the development of cardiovascular disease. As a consequence, it is vital to determine effective strategies to treat or prevent these pathologies, and accumulating evidence has implicated proinflammatory factors and the renin-angiotensin-system as promising targets in this regard. To this end, obesity is accompanied by elevated inflammation in peripheral tissues and the brain. Furthermore, obesity leads to increased levels of angiotensin-II (Ang-II), which activates central angiotensin type 1a receptors (AT1a) to influence cardiovascular function. Based on these findings and the observation that Ang-II induces hypothalamic inflammation in hypertensive models, we hypothesized that during obesity, Ang-II contributes to recruitment and activation of immune cells within the CNS which then contributes to obesity-related hypertension.
In a first set of experiments, adult male C57BL/6 mice were given either high-fat diet (HFD) or low-fat diet for 8 weeks and the effect of HFD-feeding on microglial activation, astrogliosis, and RAS activity within specific brain nuclei was examined via IHC and RT-PCR. Consumption of HFD led to an increase in the size and number of microglia within the arcuate nucleus of the

hypothalamus (ARC), the subfornical organ (SFO), the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN), but not within the magnocellular region of the PVN. Furthermore, glial fibrillary acidic protein (GFAP) immunoreactivity and gene expression were elevated within the ARC and PVN. Next, we used the Cre/lox system to delete AT1a from the PVN of mice and test the hypothesis that this population of AT1a mediates the increases in blood pressure, microglial activation and astrogliosis that are associated with obesity. Consistent with this hypothesis, PVN AT1a deletion reduced indices of hypothalamic inflammation and GFAP immunoreactivity within the PVN. In fact, although mice lacking AT1a in the PVN had an enhanced susceptibility to diet-induced obesity, deletion of AT1a from the PVN reduced systolic blood pressure, suggesting that this receptor population mediates the positive correlation between adiposity and blood pressure. Collectively, these studies demonstrate that HFD feeding initiates an AT1a-dependent inflammatory cascade within the PVN that contributes to increased blood pressure.

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Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.06/GGG12

Topic: E.04. Autonomic Regulation

Support: IRSF Help accelerate Rett Therapeutics

Title: Chronic treatment with phenytoin ameliorates the cardiac phenotype in a mouse model of Rett Syndrome

Authors: *J. A. HERRERA, JR[1,2], C. S. WARD[1,2], X. H. T. WEHRENS[2], J. L. NEUL[1,2]; [1]NRI 1250.01, Jan and Duncan Neurolog. Res. Instutite, Houston, TX; [2]Baylor Col. of Med., Houston, TX

Abstract: Eighteen percent of individuals with Rett Syndrome (RTT) have a prolonged QT (LQT) interval; a heart rhythm disorder that can lead to lethal cardiac arrhythmias. Mice deficient of Methyl CpG binding protein (MeCP2), *Mecp2*$^{Null/Y}$, recapitulate the cardiac phenotype seen in patients with RTT. The current standard therapy for patients with RTT and LQT are β adrenergic antagonist used to decrease sympathetic activity. We tested for the incidence of inducible arrhythmias with acute administration of propranolol, a β adrenergic antagonist, and did not prevent arrhythmias. Meanwhile, acute administration of phenytoin in

Mecp2$^{Null/Y}$ mice prevented induction of arrhythmias. We have designed a pre-clinical trial to investigate the physiologic and behavioral effects of chronic treatment with phenytoin or propranolol in *Mecp2*$^{Null/Y}$ mice.
Mecp2$^{Null/Y}$ and wildtype mice were randomly assigned to be treated twice a day for 28 days with either 30 mg/kg of phenytoin or vehicle. In a separate cohort, *Mecp2*$^{Null/Y}$ and wildtype mice were treated 10 mg/kg of propranolol via osmotic pump or vehicle. The experimenter was blinded for genotype and treatment. Mice were implanted with telemeters at 4 weeks and treatment was initiated soon after implantation. Programmed electrical stimulation (PES) was used to determine their susceptibility to inducible arrhythmias and to measure the electrocardiogram (ECG) for interval analysis. ECG analysis shows that *Mecp2*$^{Null/Y}$ vehicle treated mice have a prolonged QRS interval and QTc compared to the *Mecp2*$^{Null/Y}$ phenytoin treated mice. Treatment with propranolol did not correct the ECG abnormalities in *Mecp2*$^{Null/Y}$ mice. Propranolol treatment did not prevent arrhythmias in *Mecp2*$^{Null/Y}$ whereas phenytoin completely ablated arrhythmias in *Mecp2*$^{Null/Y}$ mice. Phenytoin treatment had no adverse behavior effects, and rescued the overweight phenotype in *Mecp2*$^{Null/Y}$ mice which was identified to be due to decreased abdominal fat accumulation. *Mecp2*$^{Null/Y}$ mice have a decreased basal heart rate compared to wildtype mice which was not affected by phenytoin treatment. However, treatment with phenytoin was identified to worsen the breathing phenotype seen in *Mecp2*$^{Null/Y}$ mice. Since phenytoin worsened the breathing phenotype, a more target therapy such as ranolazine should be tested to determine if it is effective in ablating arrhythmias with minimal side effects. Ranolazine has been identified to selectively block the late persistent sodium channel current which is seen in cardiomyocytes isolated from *Mecp2*$^{Null/Y}$ mice.

Disclosures: J.A. Herrera: None. **C.S. Ward:** None. **X.H.T. Wehrens:** None. **J.L. Neul:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.07/GGG13

Topic: E.04. Autonomic Regulation

Support: NHMRC 1028183

NHMRC 1030301

Title: Proteomic analysis of the caudal ventrolateral medulla in an animal model of renovascular hypertension reveals signalling and metabolic dysfunction

Authors: *A. K. GOODCHILD[1], M. MIRZAEI[1], A. DING[1], J. K. PHILLIPS[1], P. HAYNES[2]; [1]The Australian Sch. of Advanced Medicine, Macquarie Univ., Sydney, Australia; [2]Chem. and Biomolecular Sciences, Macquarie Univ., Sydney, Australia

Abstract: The caudal ventrolateral medulla (CVLM) contains cell groups that regulate blood pressure and heart rate via baroreceptor dependent and independent mechanisms, metabolism via connections with hypothalamic regions and respiration. Hypertension is present in those suffering polycystic kidney disease although the mechanisms responsible are unclear. Associated with hypertension is reduced baroreflex sensitivity which has been linked with oxidative stress (De Castro UG et al 2012) or angiotensin (Cangussu LM et al 2009) in the CVLM. The aim here is to determine what signalling pathways in the CVLM are altered in renovascular hypertension. A new Lewis rat model of polycystic kidney disease (LPK) and its genetic control were used (n=6 of each). At 12 weeks of age when severe hypertension was evident in the LPK (203 ± 15 mmHg LPK vs 100 ± 12 mmHg Lewis) the brains were rapidly removed under pentobarbitone anaesthesia and frozen. The brain was placed into a brain matrix and 1 mm coronal sections of the brain stem were taken. The CVLM region was dissected (1mm x 1.5mm) and frozen until analysis. Conventional protein preparation and digestion strategies were used and LC-MS/MS was used to identify proteins in the CVLM region of 3 LPK and 3 Lewis rats. In total 1212 proteins were identified of which 116 were differentially expressed. The distribution was relatively even with 54 proteins upregulated and 62 proteins down regulated in the LPK compared to Lewis. Key pathways were affected: Fatty acid beta oxidation was associated with changes in ACACA, FASN, ABCD3, CPT2 however this may not be linked with oxidative stress. Endocytotic changes include LAMP1, CLTA, VAC14, COP1B and EEA1. Glutamate/GABA signalling changes were associated with SLC32A1, ABAT/ DLG2, LIN7C, CAMK2A respectively. Plasticity associated changes with ARPC4, TPM4, CORO1B, ANLN, MACF1. In addition significant changes linked with GNAI3 and CNRIP1 were also present. Western blot analysis is currently being conducted. Novel targets associated with hypertension associated dysfunction could be elucidated.

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Poster

279. **Cardiovascular Regulation I**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.08/GGG14

Topic: E.04. Autonomic Regulation

Support: NHMRC GNT1030297

Title: Baroreflex control of splanchnic, renal and lumbar sympathetic nerve activity is differentially regulated in chronic kidney disease

Authors: *Y. YAO, M. M. FARNHAM, P. M. PILOWSKY, J. K. PHILLIPS;
Australian Sch. of Advanced Med., Macquarie Univ., Sydney, Australia

Abstract: The pattern of sympathetic nerve activity (SNA) to individual target organs in chronic kidney disease (CKD) is an issue that has implications for the overall hypertensive state and target organ damage. Splanchnic SNA (sSNA) controls gastrointestinal blood flow and imposes a major influence on systemic total peripheral resistance and blood pressure (BP). Lumbar SNA (lSNA) also influences BP by regulating skeletal muscle blood flow. In comparison, renal SNA (rSNA) controls long-term BP by regulating renin release and Na^+ / water reabsorption in the kidneys. Our aim was to determine if there is differential activation and regulation of SNA to these vascular beds in CKD, recording from all three nerves simultaneously and determining their respective sensitivity to baroreceptor activation. We have examined these variables using the Lewis polycystic kidney (LPK) rat model of CKD.
Under urethane anaesthesia (1.3 g/kg i.p.), 12 week old male LPK or Lewis control rats (n = 8 per experimental group) were vagotomised, paralysed and maintained on artificial ventilation. Baseline values of smoothed arterial pressure (SAP), heart rate (HR) and rSNA and lSNA and/or sSNA were recorded. Baroreflex function was assessed using a 4-parameter sigmoidal fit of SNA responses to changes in MAP, induced by intravenous infusion of phenylephrine (10-40 µg/kg) or sodium nitroprusside (50-70 µg/kg). SNA was rectified and smoothed (t = 1 s) and normalised against baseline as 100% and death level as zero.
Compared to the Lewis controls, the baseline values of SAP (80 ± 2 *vs* 88 ± 4 mmHg), rSNA (3.4 ± 0.9 *vs* 8.5 ± 2.1 µV), lSNA (2.4 ± 0.5 *vs* 9.1 ± 2.8 µV) and sSNA (3.1 ± 0.8 *vs* 6.9 ± 1.4 µ) were significantly higher in the LPK rats (all $p < 0.05$). The baroreflex functional curves for all nerve beds were shifted to the right (as reflected by MAP_{50} values) in the LPK rats [rSNA (107 ± 4 *vs* 145 ± 8 mmHg), lSNA (114 ± 4 *vs* 132 ± 5 mmHg) and sSNA (118 ± 2 *vs* 144 ± 7 mmHg), all $p < 0.05$]. Whereas the gain and maximal range of baroreflex control of rSNA and sSNA (gain 2.2 ± 0.7 vs 0.4 ± 0.05 %/mmHg & range 71 ± 7 vs 54 ± 7%, and gain 1.9 ± 0.3 *vs* 0.8 ± 0.1 %/mmHg & range 74 ± 5 *vs* 41 ± 2%, respectively, all $p < 0.05$) were significantly lower in the LPK, these parameters were not different for the baroreflex control of lSNA between strains. The elevation of basal rSNA, lSNA and sSNA in the LPK rats suggest that there is a global elevation of sympathetic tone that contributes to the hypertensive state in CKD. The maintenance of normal baroreflex responses for lSNA, despite abnormal rSNA and sSNA function suggests differential central processing of baroreflex control of SNA to different target regions.

Disclosures: Y. Yao: None. **M.M. Farnham:** None. **P.M. Pilowsky:** None. **J.K. Phillips:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.09/GGG15

Topic: E.04. Autonomic Regulation

Support: NHMRC GNT1030297

Title: Blunted pressor and renal sympathetic nerve stress response in a conscious rodent model of chronic kidney disease

Authors: *I. M. SALMAN[1], C. M. HILDRETH[1], J. L. HARRISON[2], J. K. PHILLIPS[1]; [1]Australian Sch. of Advanced Med., Macquarie Univ., Sydney, Australia; [2]Sch. of Vet. and Life Sci., Murdoch Univ., Perth, Australia

Abstract: Elevated sympathetic nerve activity (SNA) is implicated in the development of hypertension in chronic kidney disease (CKD), and is associated with increased cardiovascular morbidity and mortality. Moreover, in many hypertensive models, cardiovascular responses to acute stress are exaggerated. Using a model of CKD, the Lewis Polycystic Kidney (LPK) rat, we examined the hypotheses that these animals would show increased baseline renal SNA (RSNA) under conscious conditions, and increased cardiovascular and RSNA reactivity to acute stress. Male LPK and Lewis control rats (total n = 9) were instrumented with SNA/blood pressure transmitters (TRM56SP-Telemetry Research/Millar Instruments) at 10 weeks of age. Following a one week recovery to re-establish circadian rhythms, 5-minute recordings of mean arterial pressure (MAP) and RSNA were taken every 15 minutes for 48 hours at age 12 weeks. RSNA and haemodynamic responses to acute stress (open-field exposure for 40 minutes) were tested. The quality of the nerve signal was assessed by evaluating pulse modulation of RSNA, and ganglionic blockade with hexamethonium (20 mg/kg s.c.) was used to calculate background activity. At age 12 weeks, LPK had higher RSNA (2.8 ± 1.1 vs. 0.5 ± 0.4 μV) and MAP (150 ± 13 vs. 94 ± 2 mmHg) compared with Lewis (all $P<0.05$), thus confirming our first hypothesis. Responses to open-field stress were characterised by reproducible increases in RSNA and MAP relative to baseline levels, however the LPK showed markedly blunted sympathoexcitatory and pressor responses when compared to Lewis animals over the 40-minute stress period (RSNA: 185 ± 22 vs. 269 ± 45% and MAP: 3 ± 1 vs. 12 ± 1%, all $P<0.05$). Both RSNA and MAP had returned to baseline levels in the LPK and Lewis within 40 minutes of being removed from the open field chamber. Our demonstration of attenuated RSNA and MAP responses to acute stress were not expected. A possible explanation for these findings is that the chronic elevation in

blood pressure and RSNA observed in these animals is contributed to by endogenous stress mechanisms and therefore when these pathways are activated the relative increase is less.

Disclosures: I.M. Salman: None. **C.M. Hildreth:** None. **J.K. Phillips:** None. **J.L. Harrison:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.10/GGG16

Topic: E.04. Autonomic Regulation

Title: Computational evidence for synaptic gain in human sympathetic ganglia

Authors: *J. P. HORN, M. G. SPRINGER;
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Abstract: Extracellular recordings from conscious human subjects have revealed natural patterns of firing by postganglionic sympathetic neurons that project to limb muscles. Reports published by different investigators indicate that muscle vasoconstrictor neurons fire in bursts whose timing is coupled to the cardiac cycle through arterial baroreceptors. However, bursts only occur in a subset of cardiac cycles. Exercise increases sympathetic activity, increases burst size and increases the number of cycles that contain bursts. Elevations of sympathetic activity during hypertension, aging and heart failure show similar characteristics.
Single unit recordings indicate that neurons do not often fire repetitively during bursts, thus implying that bursts arise from recruitment of additional cells. In this study we used modeling to ask how synaptic integration at nicotinic synapses in sympathetic ganglia might contribute to this behavior. Using a similar approach, Macefield (Front Neurosci. 2011;5:132) recently concluded that human sympathetic firing could best be explained by convergence of two suprathreshold strong (i.e. primary) nicotinic synapses firing with intervals that were normally distributed. We report here that a single primary synapse is sufficient to replicate the experimental microneurography data, by simply assuming exponentially distributed intervals between synaptic events at average rates of 0.4 to 0.8 Hz. More importantly, a similar result is obtained by assuming convergence between 1 primary synapse and 6 subthreshold secondary nicotinic synapses firing at even lower rates (0.16 to 0.25 Hz). The difference in this case is that ganglionic integration results in synaptic amplification between 2.5 to 3.2. These results will be discussed in terms of the implications for baroreflex gain. The models also make predictions that

could be tested through future experiments and thereby advance the interpretation of clinical microneurographic assessment.

Disclosures: J.P. Horn: None. **M.G. Springer:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.11/GGG17

Topic: E.04. Autonomic Regulation

Support: National Space Biomedical Research Institute (SA 2002 to L.S.)

Title: Effects of G-loading and vibration on heart and breathing rate in humans

Authors: *A. GODINEZ[1], D. B. LISTON[1,2], R. AYZENBERG[3], L. S. STONE[2];
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Abstract: Operational environments commonly expose pilots and astronauts to G-loading and vibrational stress, which modulates autonomic nervous system function. Our aim was to test the effects of vibration and G-loading, alone and in combination, on heart rate (HR) and breathing rate (BR) as metrics of stress.
Methods. Observers (n=10) began each trial by fixating a central red LED. After a randomized interval (200 - 700 ms), a target spot appeared at a random eccentric location (30 deg horizontal). The task of the subject was to look at and then point to the spot location. Each subject performed this task under two G-loading (1.0 and 3.8 Gx, along the chest-to-spine axis) and four vibration (no vibration, 8, 12 16 Hz at 0.5 g zero-to-peak) conditions while wearing a physiological monitoring device (Zephyr bioharness), which recorded heart and breathing rate. Data were collected in self-paced 315-second blocks; each subject completed three blocks per experimental session.
Results. We observed a main effect of G-loading on heart ($p<0.0001$, t-test) and breathing ($p<0.05$, t-test) rate as well as a tendency for heart rate to increase over time during the 315-second task. To assess the effect of vibration frequency while compensating for the effect of performing the task, first, we fit the time-varying effect and subtracted it out for each block. Second, we analyzed the remaining HR and BR variance across frequency (2-way ANOVA). We observed a significant main effect of vibration frequency on HR ($p<0.0001$), as well as a significant interaction between vibration frequency and G-level (p0.05, 2-way ANOVA).
Conclusion. We observed strong effects of both G-loading and vibration on heart and breathing

rate, as well as interaction between G-loading and vibration. This interaction shows that the physiological impact of G-loading and vibration are not independent and can only be assessed during combined exposure to these conditions.

Disclosures: A. Godinez: None. **D.B. Liston:** None. **R. Ayzenberg:** None. **L.S. Stone:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.12/GGG18

Topic: E.04. Autonomic Regulation

Support: IBRO-SfN Travel Grants 2013 $2,000 US

Title: Effect of moderate aerobic exercise on cardiovascular autonomic function in type 2 diabetes mellitus

Authors: *R. K. GOIT, N. K. MISHRA;
Nepalgunj Med. Col., Banke, Nepal

Abstract: Aim: The aim of the study was to determine long-term cardiovascular autonomic adaptation to moderate endurance aerobic exercise in type 2 diabetes mellitus.
Methods: Heart rate variability (HRV) of 15 individuals with T2DM were assessed. Resting electrocardiogram (ECG) at spontaneous respiration was recorded for 5 min in supine position. Recording was performed before and after a nine month, supervised, progressive, aerobic training program, three times weekly. The data were expressed as median (interquartile range). The p value <0.05 was considered statistically significant.
Results: In the time domain variables, square root of the mean of the squared R-R intervals (RMSSD) [29.7 (26 - 34.5) vs. 46.4 (29.8 - 52.2) ms, p=0.02] and number of R-R intervals that differ by more than 50 ms (NN50) [35 (18 - 47) vs. 98 (20-114)] count, p=0.03) were significantly increased after exercise. In the frequency domain variables, low frequency (LF) [62.4 (59.1 - 79.2) vs. 37 (31.3-43.3) nu, p=0.03) and LF/HF [1.67 (1.44 - 3.8) vs. 0.58 (0.46 - 0.59) p=0.009] were significantly decreased while high frequency (HF) [95 (67-149) vs. 229 (98-427) power, p=0.006] and HF [37.6 (20.8-40.9) vs. 63 (56.7-68.7) nu, p=0.003) were significantly increased after exercise. In Poincare plot standard deviation (SD1) [21.3 (18.5-24.8) - 33.1 (21.5 - 37.2), p=0.027] was significantly increased after exercise.
Conclusion: These data suggest that three times weekly, nine month, moderate aerobic exercise program is associated with significant improvements in cardiovascular autonomic function in person with T2DM by increasing vagal tone and decreasing sympathetic activity.

Disclosures: R.K. Goit: None. **N.K. Mishra:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.13/GGG19

Topic: E.04. Autonomic Regulation

Support: University of Leeds

Title: Non-invasive vagus nerve stimulation reduces sympathetic nerve activity in healthy volunteers and heart failure patients

Authors: *J. A. CLANCY[1], D. A. MARY[1], K. K. WITTE[2], J. P. GREENWOOD[2], S. A. DEUCHARS[1], J. DEUCHARS[1];
[1]Sch. of Biomed. Sci., [2]Leeds Inst. of Genetics, Hlth. and Therapeut., Univ. of Leeds, Leeds, United Kingdom

Abstract: Heart failure (HF) is a debilitating and fatal condition characterised by sympathetic activation and parasympathetic withdrawal. Tackling the underlying autonomic imbalance through parasympathetic stimulation can improve cardiac function and quality of life scores in HF patients, however, the current method of cervical vagus nerve stimulation (VNS) is invasive and associated with side effects. This study investigated the autonomic effects of a non-invasive method of VNS - transcutaneous electrical stimulation of the auricular branch of the vagus nerve (tVNS) - in healthy participants (n = 34; 18 female, 16 male; aged 19-62 years) and HF patients (n = 8; 2 female, 6 male; 51-86 years). The study was approved by the University of Leeds Ethics Committee and the National Research Ethics Service and was conducted in accordance with the Declaration of Helsinki. Heart rate (ECG), blood pressure and respiration were recorded continuously. Heart rate variability (HRV) was calculated using spectral analysis of beat-to-beat intervals derived from ECG data. Microneurography was also used to record muscle sympathetic nerve activity (MSNA; n = 10). Data were analysed at baseline, during tVNS and during recovery. Repeated measures ANOVAs were used to analyse results (data presented as mean ± S.E.M). HRV improved significantly during tVNS in healthy participants ($p = 0.026$). There was a significant reduction in single unit MSNA frequency and incidence during tVNS ($p = 0.001$ and $p = 0.002$ respectively). tVNS significantly improved HRV in HF patients ($p = 0.035$). Based on the results of this study, tVNS can improve HRV in both healthy participants and HF patients. Furthermore, microneurography revealed that the improvement in HRV during tVNS is, at least partly, due to a reduction in sympathetic activity in healthy participants. tVNS could be a

practical, non-invasive and economical therapy for heart failure patients and warrants further investigation.

Disclosures: J.A. Clancy: None. **D.A. Mary:** None. **K.K. Witte:** None. **J.P. Greenwood:** None. **S.A. Deuchars:** None. **J. Deuchars:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.14/GGG20

Topic: E.04. Autonomic Regulation

Support: DFG (Berlin School of Mind and Brain)

Berlin Senate (Mind-Brain Institute)

Marie Curie Actions (CIG for Okon-Singer)

Title: Neural correlates of vascular reactivity to emotion and its interaction with attention

Authors: *L. SCHAARE[1,2], J. HOYER[1,2], J. MEHNERT[3], J. LEPSIEN[1], A. VILLRINGER[1,3], H. OKON-SINGER[4];
[1]Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Intl. Max Planck Res. Sch. NeuroCom, Leipzig, Germany; [3]Mind-Brain Inst. at Berlin Sch. of Mind and Brain, Charité and Humboldt Univ., Berlin, Germany; [4]Univ. of Haifa, Haifa, Israel

Abstract: Blood pressure and heart rate are known to change in different conditions, e.g. when performing a motor or a cognitive task or when being exposed to emotional stimuli. It is unknown how the different brain areas involved in those tasks relate to the vascular response. This open issue may be of high clinical relevance since it has recently been recognized that vascular reactivity to psychological stress predicts the risk for subsequent development of cardio- and cerebrovascular disease (Matthews et al., 2004).
We investigated vascular reactions during an emotional spatial attention task while simultaneously assessing brain activity. Specifically, we hypothesized that viewing aversive compared to neutral pictures in this task would evoke greater vascular responses and greater neural activation in corticolimbic areas, including insula, amygdala and cingulate cortex which are involved in processing of stressors and which have been suggested to be involved in mediating the vascular response. Furthermore, we expected this effect to be modulated by attention: only when stimuli were attended, there would be a difference in reactivity between

aversive and neutral information.
43 healthy volunteers (22 f, 26 ± 3.77 years) participated in one 3 Tesla fMRI session during which we recorded continuous, non-invasive blood pressure and electrocardiography (ECG). As expected, modulation of attention yielded longer reaction times, more errors and greater neural activation in visual-associated areas for attended compared to unattended conditions. Contrary to our hypotheses, these effects were more pronounced for neutral compared to aversive conditions. Vascular reactivity was modulated by attention with greater reactivity into the negative direction for aversive- compared to neutral-attended conditions and no significant reactivity difference for unattended conditions. Interestingly, ROI analyses yielded delayed peak activation (at ≈7 sec) exclusively in the left and right amygdala only when aversive stimuli were attended. A shift in time and amplitude of amygdala activation has been described in voluntary emotion regulation of aversive pictures (Walter et al., 2009). We hypothesize that the instructions to ignore the stimuli's content could have provoked similar emotion regulation processes in our participants. Thus, our results indicate that the vascular response is governed by a complex interaction of emotional content, attention, and cognitive control strategies. Understanding this interaction and its neural correlates should lead to a better understanding how vascular reactivity can contribute to the pathogenesis of vascular risk factors.

Disclosures: L. Schaare: None. **J. Hoyer:** None. **A. Villringer:** None. **J. Lepsien:** None. **J. Mehnert:** None. **H. Okon-Singer:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.15/GGG21

Topic: E.04. Autonomic Regulation

Support: Heart and Stroke Foundation of Ontario T7259

Post Doctoral Fellowship from the Canadian Institute of Health Research

Title: Sympathetic outflow in patients with obstructive sleep apnea and healthy controls correlates with grey matter thickness within the insula and cingulate cortex

Authors: *K. S. TAYLOR[1], A. J. MCREYNOLDS[1], P. J. MILLAR[1], H. MURAI[1], D. S. KIMMERLY[1], B. L. MORRIS[1], T. D. BRADLEY[1], J. S. FLORAS[1,2];
[1]Dept. of Medicine, Div. of Cardiol., Univ. Hlth. Network, Toronto, ON, Canada; [2]Dept. of Medicine, Div. of Cardiol., Mount Sinai Hosp., Toronto, ON, Canada

Abstract: Introduction: Obstructive sleep apnoea (OSA) is a pathological state characterised by exaggerated sympathetic outflow during wakefulness as well as sleep. The insula (IC) and anterior cingulate (ACC) are amongst the cortical regions considered involved in modulation of efferent sympathetic outflow but this concept has yet to be established definitively in humans. We tested the hypothesis, in patients with OSA and healthy control (HC) subjects, that grey matter (GM) thickness within the IC and ACC correlates with directly measured efferent sympathetic activity (muscle sympathetic nerve activity (MSNA)).
Methods: Seventeen OSA (3f; 58 $\pm$ 11 yrs [mean $\pm$ SD]) and 14 age-matched HC (4f; 56 $\pm$ 9 yrs) subjects underwent a 2 min resting recording of multi-unit right fibular MSNA (burst incidence (BI): bursts/100 heart beats). All subjects attended overnight polysomnography to ascertain the presence and severity of OSA. On a separate day a high-resolution FSPGR anatomical scan (FOV=24, slice thickness= 1.5mm, 120 axial slices, 256x256 matrix, flip angle=20°) was collected at 3T. A region of interest (ROI) correlation analysis between resting BI and cortical thickness within the IC and ACC was conducted using Freesurfer (ROI mask was taken from Freesurfer's built in atlas). Data was thresholded at a corrected $p \leq 0.05$ (derived from an uncorrected $p \leq 0.0075$ and 245 contiguous verticies).
Results: MSNA BI was significantly higher in OSA patients than HC (57.3$\pm$5.3 vs. 35.1$\pm$3.6 bursts/100 heartbeats respectively; $p < 0.005$). There were no significant group differences in resting heart rate, systolic, or diastolic blood pressure ($p>0.05$). The apnea-hypopnea index (average of all subjects: 19.9 $\pm$ 2.9 events/hr), a measure of OSA severity, correlated with BI ($r = 0.552$; $p \leq 0.002$). BI correlated negatively with GM in the left dorsal posterior insular cortex (LdpIC) ($r = -0.667$; $p \leq 0.001$) and correlated positively with BI in the left mid cingulate cortex (LMCC) ($r = 0.539$; $p \leq 0.002$). There were no significant correlations between BI and GM in the right hemisphere ROI.
Conclusion: The present observations are concordant with neuro-stimulatory and recent functional imaging data demonstrating insular and cingulate involvement in autonomic cardiovascular regulation, and posit contrasting roles for these ROI. They are consistent with the concepts that the left IC exerts an inhibitory and the MCC an excitatory modulator effect on MSNA. As increased sympathetic neurotransmission itself might alter regional volume, future studies should investigate the impact of long term treatment of OSA with continuous positive airway pressure, which lowers MSNA, on GM thickness within these regions.

Disclosures: K.S. Taylor: None. **A.J. McReynolds:** None. **P.J. Millar:** None. **H. Murai:** None. **D.S. Kimmerly:** None. **B.L. Morris:** None. **T.D. Bradley:** None. **J.S. Floras:** None.

Poster

279. **Cardiovascular Regulation I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 279.16/GGG22

Topic: E.04. Autonomic Regulation

Support: NSC-100-2627-B-010-002

Title: Effects of cold exposure on changes of sleep-related autonomic function and morning blood pressure surge: Human and animal studies

Authors: *C.-H. HONG[1,2], T. KUO[1,2], C. YANG[1,2];
[1]Inst. of Brain Sci., Natl. Yang-Ming Univ., Taipei City, Taiwan; [2]Sleep Res. Center, Natl. Yang-Ming Univ., Taipei City, Taiwan

Abstract: A number of studies have linked the occurrence of cardiovascular events and cold ambient temperatures as well as the morning blood pressure surge (MBPS), but findings on the effects of cold exposure on the changes of autonomic function during sleep stage transition and on the MBPS were still unclear. We hypothesized that cold exposure produce a higher sympathetic change during sleep stage transition and that this, may play an important role in cold-related cardiovascular events. In human study, all experiments were carried out on healthy male adults, who participated in two experimental conditions randomly, a warm condition (23^{o}C) and a cold condition (16 ^{o}C). Blood pressure (BP) was measured every 30 minutes for 24 hours by autonomic ambulatory BP monitoring. The electroencephalograms (EEGs), electrocardiograms (ECGs) and temperatures were recorded by miniature polysomnography over 24 hours. In animal study, polysomnographic recordings were performed on Wistar-Kyoto rats (WKYs) that were housed in thermo-regulated chambers. In humans, cold conditions led to a higher MBPS and significant sympathetic index changes during such period. The NREM-REM transition-related sympathetic elevation during the cold conditions being significantly higher in late sleep period than in early sleep period, while the vagal function index revealed contradict responses. The total power of HRV changes prior to morning awaking being significantly correlated with the changes of near body temperature and a significant higher arousal index and shorter average interval of REM periods in cold condition than in warm condition. The animal study revealed that compared with warm condition, cold conditions induced a higher BP, heart rate and sympathetic indexes and more interruptions and lower delta power. Our findings suggest that cold exposure impairs the sleep quality and elevates the amplitude of MBPS and is associated with sleep-related and late sleep stage transition sympathetic activation, which could be important implications for related cardiovascular events.
Supported by grants (NSC-100-2627-B-010-002) and (98-2314-B-010-022-MY3) from the National Science Council (Taiwan) and a grant (98001-62-006) from Taipei City Hospital.

Disclosures: C. Hong: None. **T. Kuo:** None. **C. Yang:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.01/GGG23

Topic: E.05. Stress and the Brain

Support: NIMH Grant MH080394-04

Title: Antidepressant-like effect of glucocorticoid receptor deletion in the dorsal raphé nucleus

Authors: *M. Y. VINCENT, L. JACOBSON;
Neurosci., Albany Med. Col., Albany, NY

Abstract: Glucocorticoids produced by the hypothalamic pituitary adrenal (HPA) axis can cause depression symptoms whose mechanisms remain unclear. The dorsal raphe nucleus (DRN) produces the majority of serotonin in the brain and is a target for antidepressants, which are thought to work by increasing serotonin levels in depressed patients. The DRN also expresses glucocorticoid receptors (GR). Glucocorticoids inhibit expression of the rate-limiting enzyme for serotonin synthesis in the DRN, and we have found this effect can be partially reversed by antidepressants. We therefore hypothesized that deleting GR in the DRN would have antidepressant-like effects. We used virally-transduced GR gene deletion in the DRN of floxed GR mice to test this hypothesis. We injected adeno-associated virus pseudotype AAV2/9 expressing either Cre recombinase (DRN GR KO mice) or GFP (control mice). Four weeks after injection, mice underwent tests for depression (social interaction; forced swim test; sucrose preference) in the unstressed state and again after 14d of daily social defeat stress. Before social defeat stress, deleting DRN GR increased social interactions but did not affect forced swim despair-like immobility or sucrose intake. After social defeat, deleting DRN GR had antidepressant-like effects to decrease FST despair-like behavior, increase sucrose preference and maintain higher levels of social interaction. GR DRN deletion also increased plasma corticosterone after forced swim stress. We conclude that before stress, DRN GR deletion has an antidepressant-like effect to increase social behavior, whereas during stress DRN GR may have additional roles to diminish additional depression-like behaviors and to restrain HPA activity.

Disclosures: M.Y. Vincent: None. **L. Jacobson:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.02/GGG24

Topic: E.05. Stress and the Brain

Support: R01MH086539-04 (CAL)

Title: Chronic glucocorticoid intake alters basal Tph2 protein expression in anxiety- and resilience-related midbrain serotonergic systems

Authors: *N. C. DONNER, A. MATTI, C. A. LOWRY;
Dept. of Integrative Physiol. and Ctr. for Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Stress-related psychiatric disorders, such as anxiety and affective disorders, are often correlated with disruption of hypothalamic-pituitary-adrenal (HPA) axis function and dysfunction of brain serotonergic systems. It is, however, unclear what role glucocorticoids (GCs), the primary effector stress hormones of the HPA axis, without the experience of external stressors *per se* play in the etiology of these disorders. Previous research from our lab demonstrated that chronic GC intake via the drinking water dose-dependently induces an anxiety- and depressive-like behavioral phenotype in rats, and disrupts the diurnal expression pattern of *tph2*, the gene encoding the rate-limiting enzyme for brain serotonin synthesis (tryptophan hydroxylase 2, Tph2), by increasing *tph2* mRNA expression during the rats' inactive light-phase. Chronic GC treatment has furthermore been shown to potentiate basal and stress-induced Tph activity in anxiety-related subdivisions of the midbrain dorsal raphe nucleus (DR). Here, we used western blot technique to test the hypothesis that chronic GC treatment also increases basal Tph2 protein expression. Adrenal-intact, adult male rats were treated with 100 μg/ml corticosterone (CORT) or vehicle (0.45% 2-hydroxypropyl-β-cyclodextrin) via the drinking water for 21 days, and their emotional behavior was assessed in the elevated plus-maze (EPM) and forced swim tests (FST) after 2 weeks. In concordance with previous studies, chronically GC-treated rats displayed more anxiety-like behavior on the EPM, and in tendency less proactive stress-coping behavior in the FST. In GC-treated rats, light-phase Tph2 protein expression was significantly elevated in anxiety-related subdivisions of the DR, namely the ventral (DRV) and dorsal part (DRD). Dark-phase Tph2 expression in the DR remained unaltered by GC treatment, while dark-phase (but not light-phase) Tph2 expression in the resilience-related median raphe nucleus (MnR) was in tendency ($p=0.063$) decreased after chronic GC exposure. Our results suggest that anxiety- and resilience-related brain serotonergic systems in the DR and MnR, respectively, are particularly sensitive to GC-induced alterations of Tph2 protein expression, with anxiety-related DR subdivisions being disrupted during the inactive light-phase, while the resilience-related MnR system appears to be affected during the active dark-phase. It remains to be determined whether these region-dependent effects of chronic

GC exposure on Tph2 protein expression result in altered basal and stress-induced serotonin release in specific anxiety-and resilience-related fore- and hindbrain target regions.

Disclosures: N.C. Donner: None. **A. Matti:** None. **C.A. Lowry:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.03/GGG25

Topic: E.05. Stress and the Brain

Support: NIDA DA017960 to BDW

Title: Peptidergic regulation of locus coeruleus output by local infusions of corticotropin releasing factor and the mu-opioid receptor agonist DAMGO: effects on sensory signal processing in noradrenergic terminal fields

Authors: *G. A. ZITNIK, III[1], B. D. CLARK[2], B. D. WATERHOUSE[2];
[2]Neurobio. & Anat., [1]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Corticotropin releasing factor (CRF), a neuropeptide that coordinates the cognitive and behavioral responses to stressors, activates the locus coeruleus-norepinephrine (LC-NE) system. Alternatively, mu-opioid receptor agonists, such as morphine or endogenous enkephalin, cause analgesia and sedation in part by inhibiting tonic release of LC-NE. Alterations in LC-NE output have been shown to modulate the transmission of sensory information along primary sensory pathways. The lateral geniculate nucleus (LGN) of the thalamus is the primary relay for visual signals from the retina to the cortex. In rat, the dorsal LGN (dLGN) receives noradrenergic projections derived exclusively from the LC. Using in vivo extracellular recording, we monitored single dLGN neuron activity before and after local infusions of CRF (1, 3, 10, 100 ng) or the mu-opioid receptor DAMGO, [D-Ala2, NMe-Phe4, Gly-ol5]-enkephalin, (10 pg) onto the ipsilateral LC of anesthetized rats during repeated presentation of light flashes at three different stimulus intensities. Intra-LC CRF administration reduced the latency and increased the magnitude of light-evoked responses to threshold level visual stimuli 15-60 min post-CRF for all doses tested (except 1 ng). This facilitation of light-evoked responses followed an inverted-U dose-response curve, with 10 ng eliciting the maximal increase in magnitude. There was also a dose-dependent increase in the number of cells responding to previously sub-threshold light stimulus intensities; i.e. recruiting new cells to the response pool. By contrast, intra-LC infusions of DAMGO at 10 pg, a dose that completely suppresses LC output, had no effect on response latency or magnitude of stimulus-evoked dLGN responses. These findings

suggest that different degrees of CRF-mediated LC activation prompt differential increases in NE release and subsequent dose-specific noradrenergic modulatory actions in the visual thalamus. Thus, activation of the CRF-LC-NE-stress circuit amplifies sensory signal processing by speeding the transmission of sensory signals through thalamic relay circuits and increasing the strength of such signals by magnifying threshold level responses of individual neurons to light stimuli and recruiting previously unresponsive neurons into the visual response pool. Opioid receptor suppression of LC output is believed to serve as a "braking" mechanism to return the output of the LC-NE system to near-baseline levels following CRF-mediated stressor activation. On the other hand, opioid mediated actions on basal LC firing may not manifest as detectable changes in terminal field function. (Supported by NIDA DA017960 to BDW)

Disclosures: G.A. Zitnik: None. **B.D. Clark:** None. **B.D. Waterhouse:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.04/GGG26

Topic: E.05. Stress and the Brain

Support: JSPS23590717

Title: Chronic restraint stress decreases glial fibrillary acidic protein and glutamate transporter in the periaqueductal gray matter

Authors: *H. IMBE, A. KIMURA, T. DONISHI, Y. KANEOKE;
Dept. of Physiol., Wakayama Med. Univ., Wakayama City, Japan

Abstract: Stress affects brain activity and promotes long-term changes in multiple neural systems. Exposure to stressors causes substantial effects on the perception and response to pain. In several animal models, chronic stress produces lasting hyperalgesia. The periaqueductal gray matter (PAG) is involved in various important functional activities, such as fear, anxiety, defensive reaction and autonomic regulation. It also plays a crucial role in pain modulation. The PAG receives projections from the cerebral cortices, amygdala and hypothalamus and in turn controls spinal nociceptive neurons through relays in the rostral ventromedial medulla and dorsolateral pontine tegmentum. These structures constitute neural circuit of descending pain modulatory system. Postmortem studies of stress-related psychiatric disorders have demonstrated a decrease in the number of astrocytes and the level of glial fibrillary acidic protein (GFAP), a marker for astrocyte, in the cerebral cortex. Since astrocytes play vital roles in maintaining neuroplasticity via synapse maintenance and secretion of neurotrophins, impairment of

astrocytes is thought to be involved in the neuropathology. In the present study we examined GFAP and excitatory amino acid transporter 2 (EAAT2) protein levels in the PAG after subacute and chronic restraint stresses to clarify changes in descending pain modulatory system in the rat with stress-induced hyperalgesia. Chronic restraint stress (6h/day for 3weeks), but not subacute restraint stress (6h/day for 3days), caused a marked mechanical hypersensitivity and aggressive behavior. The chronic restraint stress induced a significant decrease of GFAP protein level in the PAG (32.0±8.9% vs. control group, $p<0.05$). In immunohistochemical analysis the remarkable decrease of GFAP was observed in the ventrolateral PAG. The EAAT2 protein level in the 3weeks stress group (79.6±6.8%) was significantly lower compared to that in the control group (100.0±6.1%, $p< 0.05$). In contrast there was no significant difference in the GFAP and EAAT2 protein levels between the control and 3 days stress groups These findings suggest a dysfunction of the PAG that plays pivotal roles in organization of strategies for coping with stressors and in pain modulation after chronic restraint stress.

Disclosures: H. Imbe: None. **A. Kimura:** None. **T. Donishi:** None. **Y. Kaneoke:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.05/GGG27

Topic: E.05. Stress and the Brain

Title: Effect of different corticosterone administration methods on corticosterone serum levels and forced swim test behavior in female rats

Authors: F. A. SHOUBAH, J. M. KOTT, *S. BRUMMELTE;
Psychology, Wayne State Univ., Detroit, MI

Abstract: High levels of chronic stress or stress hormones are associated with depressive-like behavior in animal models. However, slight elevations in corticosterone -the major stress hormone in rodents- have also been associated with improved performances, albeit in a sex-dependent manner. Some of the discrepancies in the literature regarding the effects of high corticosterone levels may be due to different administrations methods. The current study aims to compare the effects of ~ 40mg/kg given either via subcutaneous injection, through an implanted pellet, or via the drinking water for 21 days on depressive-like behavior in the forced swim test, as well as on diurnal corticosterone serum levels in adult female rats. We hypothesize that animals exposed to the daily injections will show the highest corticosterone levels by the end of the administration period and will still show diurnal differences, while the other groups may

have diminished serum concentrations as well as a flattened diurnal rhythm. In addition, we expect the injection group to show higher levels of immobility in the forced swim test. The results will contribute to the growing literature on the effects of chronic corticosterone exposure and may help to clarify some of the discrepancies among previous studies.

Disclosures: F.A. Shoubah: None. **J.M. Kott:** None. **S. Brummelte:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.06/GGG28

Topic: E.05. Stress and the Brain

Support: NIH Grant MH072672

NIH Grant MH053851

NARSAD Yioung Investigator Grant 44096

Title: Ketamine restores active coping behavior after stress in the rat defensive burying test - Evidence for a role of STAT3

Authors: *M. GIROTTI[1], J. J. DONEGAN[2], E. A. FUCICH[2], D. A. MORILAK[2]; [1]Pharmacol., Univ. Texas Hlth. Sci. Ctr., SAN ANTONIO, TX; [2]Pharmacol., Univ. of Texas Hlth. Sci. Ctr. San Antonio, San Antonio, TX

Abstract: Coping style influences the psychological and physiological burden of stress and there is evidence from both clinical and preclinical studies that active coping is more adaptive than passivity. Defensive burying (DB) refers to the innate rodent behavior of displacing bedding material towards a source of threat, such as an electrified probe placed inside a test cage. Because DB is a well-validated model of defensive behavior that includes both passive (immobility) and active (burying) behaviors in response to the stressful experience of the shock, it may provide a useful context to study molecular and cellular mechanisms of shifts in coping styles. Burying behavior in the rat is dependent on circuitry from the lateral septum, prefrontal cortex, hippocampus and nucleus accumbens. We have previously shown that noradrenergic neurotransmission in the lateral septum modulates burying behavior and that a stress paradigm designed to model aspects of post-traumatic stress disorder shifts the coping style from active to passive behavior.
In this study we have examined whether other types of chronic stress paradigms influence the

choice of behavior in the defensive burying test and we have begun an investigation of the possible underlying mechanism. First, we tested whether 2 weeks of chronic intermittent cold (CIC) stress or chronic unpredictable stress (CUS) might shift the coping style from active (burying behavior) to passive (immobility) in the DB test. We found that both CIC and CUS animals displayed significantly less burying behavior than control rats. CUS also produced a significant increase in immobility, indicating that CUS shifts the rat's coping style from active to passive. We then investigated whether ketamine, a NMDA receptor antagonist previously shown to revert behavioral and cellular effects of stress, might reduce the CUS-driven increase in passive behavior. We found that ketamine (10mg/kg, i.p., 24h prior to behavioral testing) shifted the behavior of CUS animals from passive to active (decreased immobility and increased burying), indicating that ketamine-induced plasticity affects the choice of behavior in this test. We are currently searching for the molecular correlates of the behavioral shifts produced by stress and ketamine. Initial data with hippocampal sub-cellular fractions show that ketamine increases phosphorylated STAT3 in the nucleus. We have found in other studies that STAT3 is implicated in cognitive flexibility; the current results suggest it may also have a role in the ketamine-driven plasticity that underlies coping style choice.

Disclosures: M. Girotti: None. **J.J. Donegan:** None. **E.A. Fucich:** None. **D.A. Morilak:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.07/GGG29

Topic: E.05. Stress and the Brain

Support: The Defense Advanced Research Projects Agency, U. S. Army Research Laboratory and the U. S. Army Research Office under contract/grant number W911NF101006

Title: Phenotypic relationships among multiple measures of stress reactivity

Authors: *K. FITZPATRICK, P. JIANG, M. H. VITATERNA, F. W. TUREK;
Ctr. for Sleep and Circadian Biol., Northwestern Univ., EVANSTON, IL

Abstract: Resilience to stress is fundamentally required in order to maintain mental and physical health, although research into the basis of resilience has been largely restricted by the use of a limited number of stressors, response measurements, and number of subjects. We set out to identify specific molecular and genetic mechanisms underlying the stress response and resilience capacity in a comprehensive and high-throughput fashion. We utilized eight different stressors, both physical and emotional in nature, as well as a comprehensive panel of response

measurements including behavioral, cognitive, hormonal, immune, molecular, and sympathetic nervous system response systems. Here, we will present data from these multiple phenotypic measurements collected throughout a 8-week long chronic stress protocol in a genetically segregating population of 297 male (C57BL/6J x A/J) F2 mice.
We identified several correlational relationships among the phenotypic measures which revealed both expected and unexpected associations. For example, both body weight and blood glucose levels were measured at four timepoints throughout the chronic stress protocol, and we found that these two interrelated measures were significantly correlated under predominantly physical stress conditions. However, this significant relationship was not present when the stress was emotional in nature, as in the case of social defeat. Additionally, corticosterone levels at the time of necropsy correlated significantly with the naïve measure of anxiety behavior, but did not demonstrate a relationship with any of the intermediary corticosterone measurements under both physical and emotional stressors. Furthermore, we found that various behavioral measures of anxiety taken throughout the protocol were significantly correlated. Together, the relationship between and among baseline corticosterone levels and outward anxiety behaviors suggests an underlying stability of stress resistance, possibly of genetic origin. We also utilized factor analysis to uncover underlying structure among the measures, which identified four factors grouped as metabolic function, fearfulness, glucocorticoid activity, and anxiety, and which explain approximately 25% of the variance within the dataset.
We are currently analyzing how these phenotypic measures relate to genotype and gene expression levels in various brain regions in order to identify specific genetic networks for future study in stress and for the development of novel therapies to prevent stress-related decrements in the brain and cognitive performance.

Disclosures: K. Fitzpatrick: None. **P. Jiang:** None. **M.H. Vitaterna:** None. **F.W. Turek:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.08/GGG30

Topic: E.05. Stress and the Brain

Support: National Basic Research Program of China (2013CB531906)

Title: Anti-stress effects of electro-acupuncture (EA) on the expression of corticotropin-releasing factor (CRF) and GABA receptors in the hypothalamus and hippocampus in acute stress rats<!--EndFragment-->

Authors: *L.-T. ZHU, Y. FENG, G.-C. WU, Z.-Z. TIAN;
Fudan Univ., Shanghai, China

Abstract: CRF is a hypothalamic releasing peptide that regulates the endocrine, autonomic and behavior response to stress through interaction with typical neurotransmitters. In stress response, the CRF in the paraventricular nuclei (PVN) is signaled by the amygdala and then releases CRF to activate the HPA axis and also interacts with the hippocampus, which may suppress the hyperactivity of HPA axis through the interaction with the activation of GABA receptors. As demonstrated by many previous studies, EA treatment of acute stress is associated with the amygdaloid, hypothalamic CRF expression and regulation of the HPA axis. It is still unknown whether EA exerts central effects between hypothalamus and hippocampus in acute stress. The acute stress rats was provoked by partial hepatectomy. The righting reflex was examined after surgery immediately or 30min after anesthesia in rats without surgery. Hypothalamic and hippocampal CRF, GABA receptors levels were measured using the methods of western blot and real-time PCR. Statistically significant acceleration was observed in the recovery time over the period of that EA treatment (Fig.1). EA also decreased hypothalamic CRF mRNA and CRF1 receptor mRNA levels, while increasing GABA(A) α3 and α4 subunit mRNA levels. The hippocampal GABA(A) α5 subunit mRNA was decreased after EA treatment.(Fig.2). The hypothalamic CRF, α1 and α3 subunit of GABA(A) receptor and GABA(B) receptor were also found decreased by EA treatment. But hippocampal CRF and Ucn levels were increased. However, no statistically significant differences were observed in the hippocampal GABA receptors and GR (Fig.3). The aim of the study focuses on the suppression effect of hippocampal CRF neurons on hypothalamic CRF activation and anti-stress effects of EA. These data demonstrate that EA may attenuate the acute stress by modulating hippocampal CRF so that to activate the hypothalamic GABA receptors to inhibit the CRF neurons in the hypothalamus.

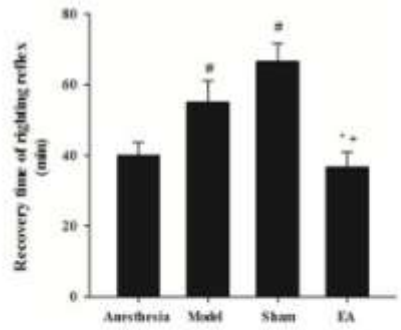

Fig 1. Effects of EA on the recovery of righting reflex after operation
The recovery time was measured every 10 min after the surgery. The operative rat with sham EA and without treatment showed long time recovery. But EA treatment promote the recovery time after the hepatectomy.
#, versus anesthesia; *, versus model; +, versus sham (acupuncture without electric)

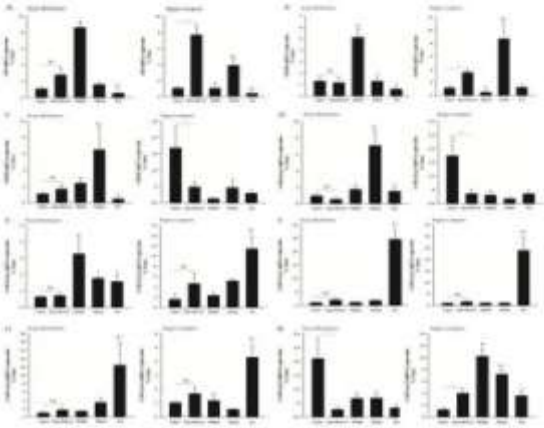

Fig 2. The mRNA level of CRF and its receptors, GABA(A) receptors expression changes after EA.
In acute stress, hypothalamic CRF, CRF1 receptor and GABA(A) α2 subunit mRNA were increased after surgery, but GABA(A) α5 was decreased, which was opposite to the changes of the hippocampus. However, despite of the down regulated CRF, CRF1 receptor and α2 subunit mRNA, EA treatment also up regulated the α3 and α4 subunit in the hypothalamus, increased the α2, α3 and α4 subunit and decreased α5 subunit in the hippocampus.
#, versus anesthesia; *, versus model; +, versus sham.

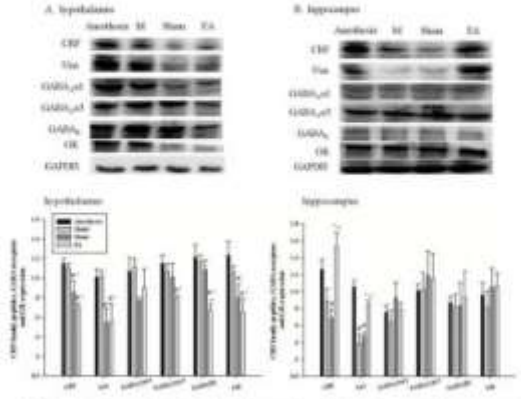

Fig 3. The anti-stress effects of EA on the expression of CRF, glucocorticoids receptor and GABA receptors.
At the protein level measured by western blot, both sham EA and true EA decreased CRF, urocortin and glucocorticoids receptor expression in the hypothalamus. But only EA treatment decreased hypothalamic α3 subunit and GABA(B) receptor level, and increased the hippocampal CRF expression.
#, versus anesthesia; *, versus model; +, versus sham.

Disclosures: L. Zhu: A. Employment/Salary (full or part-time):; no. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Basic Research Program of China (2013CB531906). C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); no. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); no. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); no. F. Consulting Fees (e.g., advisory boards); no. Other; no. **Y. Feng:** None. **G. Wu:** None. **Z. Tian:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.09/GGG31

Topic: E.05. Stress and the Brain

Support: NIH/NIAAA

NSERC

Title: Time-dependent increase in cortisol in response to an acute stressor and the role of the dopaminergic and serotoninergic system in zebrafish (Danio rerio)

Authors: *S. TRAN[1], D. CHATTERJEE[2], R. GERLAI[2];
[1]Cell and Systems Biol., [2]Psychology, Univ. of Toronto, Mississauga, ON, Canada

Abstract: The zebrafish stress response is characterized by the release of cortisol from the interrenal gland, similar to the release of cortisol from the adrenal gland in humans. However, the use of zebrafish in behavioural neuroscience is still novel with little information on the hypothalamic-pituitary-interrenal axis. In the current experiment we exposed zebrafish to an acute net handling stressor (30 seconds) and quantified cortisol and neurochemical levels at 0, 1, 5, 10, and 15 minutes post-stressor. A separate group of zebrafish were immediately sacrificed without the stressor to serve as controls. Whole body cortisol levels were then quantified at each

time point by ELISA using a human saliva cortisol kit. In addition, the levels of dopamine, 3, 4-dihydroxyphenylacetic acid, serotonin, and 5-hydroxyindoleacetic acid from whole-brain samples were quantified by using high precision liquid chromatography (HPLC). Results demonstrated that whole body cortisol levels increased in a time-dependent manner with levels peaking at 15 minutes. Interestingly, there were no significant differences in any of the neurochemicals measured in response to stress. Previous findings showed the dopaminergic and serotoninergic system to respond to specific environmental stimuli (animated computer images) within minutes after the exposure. Our current findings together with those already published suggest that hormonal measures such as cortisol may be more sensitive to experimenter handling prior to decapitation, whereas neurochemical measures may be more resilient to this particular manipulation. The findings here provide a possible explanation for the commonly found larger variance in cortisol levels in zebrafish compared to neurochemical data.

Disclosures: S. Tran: None. **D. Chatterjee:** None. **R. Gerlai:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.10/GGG32

Topic: E.05. Stress and the Brain

Support: CIHR

NSERC

CCIC

Title: Adolescent cannabinoid treatment sex dependently alters adult stress responsivity

Authors: *T. T.-Y. LEE[1], S. R. WAINWRIGHT[2], M. N. HILL[5], L. A. M. GALEA[3], B. B. GORZALKA[4];
[1]PSYCHOLOGY, Univ. of British Columbia, VANCOUVER, BC, Canada; [2]Neurosci., [3]Psychology, Neuroscience, Brain Res. Ctr., Univ. of British Columbia, Vancouver, BC, Canada; [4]Psychology, Univ. of British Columbia, vancouver, BC, Canada; [5]Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: Cannabinoid exposure during adolescence has been shown to have a variety of adverse effects on neuroplasticity, emotional behaviour, cognition and reward sensitivity in adult rats. We investigated whether escalating doses of the cannabinoid CB1 receptor (CB1R) agonist,

HU-210, in adolescence would affect adult stress responsivity to a 30 min restraint session in male and female Sprague-Dawley rats. Escalating doses of HU-210 (25, 50, and 100 µg/kg), or vehicle were administered from postnatal day (PND) 35 to 46. Animals were left undisturbed until PND 75, in which hypothalamic-pituitary-adrenal (HPA) axis reactivity to an acute restraint stress (30 min; PND 75) was assessed. Female rats, regardless of adolescent treatment condition, had higher corticosterone levels than males at 0, 30, and 60 min following restraint stress onset. Adolescent HU-210 administration induced significantly higher peak corticosterone levels despite comparable basal levels. This effect was more pronounced in males than females when comparing the relative corticosterone response to vehicle injected groups. These findings were surprising given that females are generally known to exhibit stress sensitivity. Results of this study indicate increased CB1 receptor activation during adolescence results in long-term, sex dependent alterations to HPA axis stress responsivity in male, but not female rats. Further studies are necessary to determine the mechanisms underlying these differential effects.

Disclosures: T.T. Lee: None. **S.R. Wainwright:** None. **M.N. Hill:** None. **L.A.M. Galea:** None. **B.B. Gorzalka:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.11/GGG33

Topic: E.05. Stress and the Brain

Title: Orexin-1 receptor antagonism attenuates stress-induced arousal in rodents

Authors: S. YUN, J. SHELTON, P. BONAVENTURE, B. SHIREMAN, T. LOVENBERG, *C. DUGOVIC;
Janssen Res. & Develop., San Diego, CA

Abstract: Orexins are peptides produced by lateral hypothalamic neurons that exert a prominent role in arousal-related process including stress by activating orexin-1 (OX1R) and orexin-2 (OX2R) receptors located widely throughout the brain. Selective pharmacological blockade of OX2R promotes sleep by inhibiting the output of wake active neurons in the hypothalamus and brainstem regions. In contrast, pharmacological or genetic selective inhibition of OX1R does not affect sleep. Stress and corticotrophin-releasing factor activate orexin neurons, and orexin administration stimulates hyperarousal, ACTH and corticosterone release. More recently, it has been shown that in several rodent models of panic anxiety disorder activation of orexin neurons could be specifically mediated by OX1R, as OX1R antagonism can block the panic responses. In

the present study, we first evaluated the arousal effects of a psychological stress elicited by cage exchange (animal placed in a dirty cage previously occupied by another animal for one week) on EEG sleep, locomotor activity in rats, and on pituitary-adrenal activation in mice. In this model, we tested whether a selective OX1R antagonist (compound A) could attenuate these behavioral activating effects. In mice, cage exchange-induced stress produced a significant increase in ACTH plasma levels consistent with an activation of the HPA axis. In rats, cage exchange stress induced a significant delay in both non-REM and REM sleep latencies associated with an increase in wake duration and locomotor activity as compared to the control condition (brief handling). Administration of the OX1R antagonist alone did not affect any EEG sleep-wake parameters either in baseline or in control condition compared to vehicle-treated rats, but prevented the sleep onset insomnia (i.e. prolonged non-REM and REM sleep latencies) and partially attenuated the active wake induced by cage exchange. These data indicate that in a psychological stress model, behavioral arousal responses can be attenuated by a selective OX1R antagonist that could represent a new therapeutic strategy for anxiety disorder without affecting sleep per se.

Disclosures: S. Yun: None. **J. Shelton:** None. **P. Bonaventure:** None. **B. Shireman:** None. **T. Lovenberg:** None. **C. Dugovic:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.12/GGG34

Topic: E.05. Stress and the Brain

Support: NIH Grant DE021888

Title: Toll-like receptor 4-mediated nuclear factor kappa B activation is essential for sensing exogenous oxidants to propagate and maintain oxidative/nitrosative cellular stress

Authors: *O. J. IGWE, R. KARKI;
Div. of Pharmacol. & Toxicology, Univ. Missouri- KC, KANSAS CITY, MO

Abstract: Objectives: To determine the mechanism(s) by which cells may sense exogenous oxidants to potentially initiate, propagate and/or maintain inflammation through NF-κB activation.
Methods: First, we used a human embryonic kidney (HEK-Blue) cells that stably express either mouse toll-like receptor 4 (TLR4) with the CD14/MD-2 co-receptor genes, or TLR2 with the CD14 co-receptor gene against a null background. These cells were used to examine the role of

pro-oxidants on TLR4- and TLR2- dependent NF-κB activation. Cells also express optimized secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of a promoter inducible by NF-κB transcription factor. These cells were challenged with their respective receptor-specific ligands, different pro-oxidants and /or inhibitors that act at different levels of TLR4 or TLR2 signaling pathways. We used the level of SEAP released into the culture media due to NF-κB activation as a measure of the extent of TLR4 or TLR2 stimulation. To integrate the *in vitro* system with an *in vivo* model, we used an orofacial behavioral pain model. We investigated the role of a novel prooxidant in regulating the expression of TLR4 and the levels of proinflammatory cytokines in the mouse masseter (masticatory) muscle.
Results: Pro-oxidants evoked increased release of SEAP from HEK-Blue mTLR4 cells at a much lower concentration compared with release from the HEK-Blue mTLR2 cells. Specific TLR4 signaling pathway inhibitors and oxidant scavengers (anti-oxidants) significantly attenuated oxidant-induced SEAP release by TLR4 stimulation. A neutralizing antibody directed against TLR4 inhibited responses to both TLR4-specific agonist and a prooxidant, which confirmed that both TLR4-specific and prooxidant act through TLR4. Furthermore, a novel pro-oxidant that decays to produce the same reactants as activated phagocytes induced inflammatory pain responses in the mouse orofacial region with increased TLR4 expression, and IL-1ß and TNFα tissue levels. EUK-134, a synthetic serum-stable scavenger of oxidative species decreased these effects. Pro-oxidants activate NF-κB by interacting mainly with TLR4.
Conclusions: Exogenous oxidants stimulated TLR4 at a much lower concentration than TLR2. Oxidant-induced orofacial pain increased TLR4 expression that was reduced by a scavenger for oxidative species. Our data provide *in vitro* and related *in vivo* evidence that exogenous oxidants can induce and maintain inflammation by acting mainly through a TLR4-dependent pathway.

Disclosures: O.J. Igwe: None. **R. Karki:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.13/GGG35

Topic: E.05. Stress and the Brain

Support: JDRF

DK-45735

ECRIP

CWPW

Title: Cytisine (partial nicotinic receptor agonist) can alter epinephrine responsiveness to insulin-induced recurrent hypoglycemia

Authors: *B. B. NANKOVA[1], N. KIRTOK[2], U. AKPAN[2], O. CHAN[3], E. LAGAMMA[4]; [1]Pediatrics & Biochem & Mol Biol, New York Med. Col., Valhalla, NY; [2]Pediatrics, The Regional Neonatal Center, Maria Fareri Children's Hosp. at Westchester Med. Ctr., Valhalla, NY; [3]Intrnl. Medicine, Endocrinol., Yale Sch. of Med., New Haven, CT; [4]Pediatrics & Biochem & Mol Biol, New York Med. College, and Regional Neonatal Center, Maria Fareri Children's Hosp., Valhalla, NY

Abstract: Recently we have shown that depending on the daily frequency of stress episodes recurrent hypoglycemia can either increase adrenal TH mRNA and protein (once daily) to sustain biosynthesis and release of epinephrine or, paradoxically, suppress it's induction (twice daily) resulting in depletion of adrenal catecholamine stores and progressive loss in circulating epinephrine. Since both, the release and the synthesis of epinephrine are mediated by adrenal nicotinic acetylcholine receptors (nAchR); we hypothesized that their excessive stimulation during recurrent twice daily episodes of hypoglycemia contributes to the impaired counter-regulation.

The plant alkaloid cytisine is a nicotinic receptor partial agonist primarily studied for its effect on selected subtypes of central nAChR. Its effect on peripheral neuronal nAChRs and the attendant autonomic neurotransmission has not been addressed. Here we demonstrate that cytisine acts as a partial agonist of adrenal nAchRs *in vitro* (PC12 cells) and *in vivo* (male, non-diabetic SD rats) in regard with two main biological outputs: release of neurotransmitters and increase in catecholamine biosynthesis as compared to nicotine or endogenous neurotransmitter (acetylcholine released in response to hypoglycemia). In addition, when animals were pre-treated with cytisine, epinephrine responses to acute hypoglycemia were reduced by 30%. Interestingly, when given before each episode of twice daily recurrent hypoglycemia (an animal model of hypoglycemia associated autonomic failure, HAAF) cytisine improved the counter-regulatory responses. As hypothesized, the changes in TH mRNA levels correlated with the magnitude of the corresponding epinephrine responses. These experiments provide a proof of concept, that pharmacological manipulation of adrenal nAchR can improve the attenuated epinephrine counter-regulation *in vivo* in an animal model of HAAF and thus offer promise as a translational adjunctive therapy for insulin-dependent diabetes.

Disclosures: B.B. Nankova: None. **N. Kirtok:** None. **U. Akpan:** None. **O. Chan:** None. **E. LaGamma:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.14/GGG36

Topic: E.05. Stress and the Brain

Support: CIHR

Michael Smith Foundation for Health Research

Title: Repeated restraint stress alters the corticosterone response to serotonin 1A receptor activation in males, but not females

Authors: *N. GOEL, V. VIAU;
Cell. and Physiological Sci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Maladaptive stress coping is associated with increased risk of affective disorders such as depression and anxiety. These disorders are more prevalent in women than in men and involve dysfunction of both the serotonin (5-hydroxytryptamine, 5-HT) system and the hypothalamic-pituitary-adrenal (HPA) stress axis. Alterations in 5-HT 1A receptor function could contribute to this sex disparity, as depression in humans and chronic stress in rodents are associated with blunted HPA axis responses to systemic administration of 5-HT 1A receptor agonists. Using a model of stress habituation, our current findings indicate that female rats do not show a reduced corticosterone (CORT) response to restraint stress after the fifth exposure, while males show robust attenuation. We propose that this sex difference in HPA axis habituation could be related to differential changes in 5-HT 1A receptor function. To investigate this possibility, we exposed adult male and female rats to 5 daily episodes of 2 hour restraint stress. We then examined HPA output responses to the selective 5-HT 1A receptor agonist, 8-OH-DPAT (0.2 mg/kg, sc), 24 h after the last restraint exposure. Again, our results showed that the CORT response to the fifth day of restraint stress compared to the first day is reduced in males, but not in females. Compared to unstressed animals, male rats exposed to repeated restraint showed an enhanced CORT response to 8-OH-DPAT. In contrast, a history of repeated stress in females had no impact on the response to 8-OH-DPAT. Previous studies show that the HPA response to acute 8-OH-DPAT administration is mediated by postsynaptic 5-HT 1A receptors. Thus, the present results provide insight into the mechanisms involved in stress adaptation and foreshadow sex-dependent changes in 5-HT 1A receptor function within forebrain afferents to the HPA axis.

Disclosures: N. Goel: None. **V. Viau:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.15/GGG37

Topic: E.05. Stress and the Brain

Support: SAF 2009-08319

Title: Partial insulin-like growth factor 1 (igf-1) deficiency promotes cerebral oxidative damage

Authors: J. E. PUCHE, U. MUÑOZ, L. GUERRA, *I. CASTILLA DE CORTAZAR;
Univ. CEU San Pablo, Madrid, Spain

Abstract: Background: Insulin-like growth factor-1 (IGF-1) is a polypeptide hormone responsible for a wide range of systemic activities (anabolic, cytoprotective and anti-inflammatory). In the central nervous system, it has been documented a relevant role for IGF-1 in normal neuron progenitor proliferation and differentiation, neuritic outgrowth, and amyloid-β clearance, among others. A model of partial IGF-1 deficiency may shed light into these underlying mechanisms.
Material and Methods: Heterozygous (*Igf1*$^{+/-}$) 5 months old mice (Hz, n=10) were compared to homozygous *Igf1*$^{+/+}$ (WT, n=10, same age) by assessing cerebral gene expression (via quantitative PCR) of IGF-1-related factors, antioxidant enzymes, inflammatory response components, heat shock proteins and cell death markers. The effect of the replacement therapy with low doses of IGF-1 (2 µg/100 g body weight/day, for 10 days, s.c.) was evaluated in parallel (group Hz+IGF, n=10, same age). Complementarily, magnetic resonance images (both ADC and MT maps) were obtained from these mice brain.
Results: We firstly confirmed that the reduced *Igf1* gene expression correlated with low circulating levels of this hormone what, in turn, induced a lower body weight of IGF-1 deficient mice. Cerebral expression of IGFBPs was found altered for isoforms 4, 6, 7 and 8. IGF-1 receptor gene expression was increased in Hz mice, thus suggesting a compensating mechanism due to the lower IGF-1 levels. An abnormal gene expression of antioxidant enzymes (*Cat* and *Gpx*), inflammatory response components (interleukins and *Ptgs2/Cox2*), heat shock proteins (*Hsp90b1, Hsp13, Hsp1* and *Hspa2*), and cell death markers (*Gadd45a, Ulk1, Grb2, Bid* and *Parp1*) was also observed in Hz mice. Substitutive IGF-1 was able to restore all of these factors. Finally, ADC and MT maps reported signs compatible with edema and inflammation, what has been previously associated with neuronal loss and gliosis.
Conclusion: This model of IGF-1 deficiency unravels protective cerebral actions of IGF-1 in adult mice, since the mere IGF-1 deficit (with no other external damage) increases parameters of oxidative damage, inflammation and cell death, that IGF-1 replacement therapy reduces to those values found in control animals.

Disclosures: J.E. Puche: None. **U. Muñoz:** None. **L. Guerra:** None. **I. Castilla De Cortazar:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.16/GGG38

Topic: E.05. Stress and the Brain

Support: 58077LSDRP (DARPA)

Title: The use of designer receptors exclusively activated by designer drugs (DREADDS) to elucidate the role of orexins in adaptations to stress

Authors: *S. BHATNAGAR[1], A. SENGUPTA[2], W. HEYDENDAEL[2], J. PEARSON-LEARY[2], G. KELLY[2], D. PIEL[2], S. BECK[1];
[1]Dept Anesthesiol., Univ. Pennsylvania, Children's Hosp Philadelphia, PHILADELPHIA, PA;
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Abstract: Maladaptation to repeated stress is associated with psychopathology. However, our understanding of the underlying neural circuitry involved in adaptations to repeated stress is limited. Previous work from our lab has indicated an important role for the hypothalamic neuropeptides orexins in regulating the neuroendocrine and behavioral responses to repeated stress. To further elucidate how orexins regulate stress-induced behaviors, we employed a pharmacogenetic technique of evolved G protein coupled receptors (also known as DREADD) that can be activated by administration of the otherwise inert clozapine-n-oxide (CNO) to manipulate orexin neurons in vivo. We generated adenoviruses (AAV) encoding either the Gq-coupled hM3Dq or Gi-coupled hM4Di receptor fused to mCitrine under the control of the prepro-orexin promoter and injected them bilaterally to the lateral hypothalamus, the site of orexin cell bodies. Expression of these constructs was verified by co-immuno labeling with GFP and OrexinA. The functionality of the constructs was verified by bath application of CNO to hypothalamic slices using whole cell patch clamping of mCitrine-expressing cells to record baseline and CNO-evoked electrophysiological responses. Cells expressing hM3Dq exhibited increased firing whereas cells expressing hM4Di exhibited hyperpolarization after CNO application. We then examined whether repeated orexin activation or inhibition using DREADDs affected behavior during repeated defeat. We have previously shown that variations in average latency to be defeated during 7 days of defeat in the resident-intruder paradigm are associated with distinct behavioral consequences. Animals exhibiting short latencies to be defeated are passive and exhibit increased anxiety- and depression-related behaviors. In contrast, animals exhibiting long latencies to be defeated exhibit active coping strategies including increased upright boxing postures. AAV-injected rats (n=6 hM3Dq, n=4 hM4Di) were exposed to 5 days

of social defeat with no drug to establish their latency profiles followed by 3 days of social defeat with CNO application 1 hour prior to each of the three days of defeat. We found that inhibiting the orexin cells (hM4Di) significantly reduced ($p \leq .03$) their average defeat latency. In contrast, stimulating orexin cells (hM3Dq) significantly increased ($p \leq .03$) their average defeat latency compared to the 5 days of defeat without CNO application. These results suggest that orexin neurons play an important role in behavioral coping strategies exhibited during stress.

Disclosures: S. Bhatnagar: None. **A. Sengupta:** None. **W. Heydendael:** None. **J. Pearson-Leary:** None. **G. Kelly:** None. **D. Piel:** None. **S. Beck:** None.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.17/GGG39

Topic: E.05. Stress and the Brain

Support: NIH Grant R01MH086539

Title: Noise stress increases *In vivo* tryptophan hydroxylase activity in serotonergic systems and within anxiety and emotion related brain regions

Authors: *J. H. FOX[1], E. D. PAUL[1], B. M. SPANNUTH[1], M. W. HALE[2], C. A. LOWRY[1]; [1]Dept Integrative Physiol., Univ. of Colorado, Boulder, CO; [2]Sch. of Pyschological Sci., La Trobe Univ., Melbourne, Australia

Abstract: Studies using the acoustic startle paradigm have reported changes in serotonergic systems arising from the midbrain raphe complex. A pure tone used as a noise stressor increases in vivo tryptophan hydroxylase (TPH) activity, as measured by 5-hydroxytryptophan (5-HTP) accumulation following administration of the aromatic amino acid decarboxylase inhibitor NSD-1015 in the median raphe nucleus (MnR). More recent studies in our laboratory reported that acoustic stimuli increased in vivo TPH activity selectively within the caudal part of the dorsal raphe nucleus (DRC). Further, acoustic stimuli increase corticosterone levels which may be necessary for the increased TPH activity as adrenalectomized rats do not show the increase in TPH activity induced by acoustic stimuli. As most studies investigating the acoustic startle response use white noise as a startle stimulus, we investigated whether white noise as opposed to a pure tone would also induce an increase in TPH activity in midbrain serotonergic systems. We measured 5-hydroxytryptophan (5-HTP) accumulation in male Sprague Dawley rats as an index of TPH activity following inhibition of aromatic amino acid decarboxylase (using NSD-1015). Treatment groups included home cage control (HCC), exposure to a startle chamber with no

noise (sham-stress; AS−), exposure to a pure tone stimulus (30 tones over 30 min with a variable ITI; 3.0 kHz; 100 ms; 100 dB; AS1) and exposure to a white noise stimulus (30 tones over 30 min with a variable ITI; 3.0-32.0 kHz; 50 ms; 100 dB; AS2) group. We replicated previous findings reporting pure tones, relative to HCC, increase 5-HTP in the MnR as well as other anxiety- and emotion-related brain regions, including the cingulate cortex, area 1 (Cg1), infralimbic cortex (IL), accumbens nucleus, shell (AcbSh), dorsal hypothalamic area (DA)/posterior hypothalamic area (PH), and dorsal raphe nucleus, ventral part (DRV). In contrast, white noise had no effect on 5-HTP accumulation in the midbrain raphe complex, but increased 5-HTP within anxiety- and emotion-related brain regions like the Cg1 and IL. Importantly, we also found that sham-stress increased 5-HTP accumulation in the DRV, IL and AcbSh, relative to HCC conditions, suggesting that some of the effects of noise stress per se could be accounted for by the stress of being restrained in the chamber. These data indicate separate but related brain regions involved in specific types of noise stress. In addition, some of the effects of the noise stress paradigm can be accounted for by acute mild restraint stress-induced increases in in vivo tryptophan hydroxylase activity.

Disclosures: J.H. Fox: None. **E.D. Paul:** None. **B.M. Spannuth:** None. **M.W. Hale:** None. **C.A. Lowry:** F. Consulting Fees (e.g., advisory boards); Enlight Biosciences.

Poster

280. **Stress-Modulated Pathways: Brainstem and Other Responses to Stress**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 280.18/GGG40

Topic: E.05. Stress and the Brain

Title: Cortisol expression and related behavior change in the zebrafish (danio rerio)

Authors: *D. J. ECHEVARRIA[1], A. D. COLLIER[1], D. J. JOUANDOT[2], C. BREAZEALE[3]; [1]Dept Psychol, Univ. Southern Mississippi, HATTIESBURG, MS; [2]Brother Martin High Sch., New Orleans, LA; [3]Southern Illinois Univ., Carbondale, IL

Abstract: The zebrafish (Danio rerio) animal model is steadily gaining popularity in behavioral neuroscience research. This species' stress response has been reported to induce observable behavioral alterations within a number of paradigms adopted from the rodent literature. In this study, zebrafish were exposed to an acute stressor, consisting of isolation and confinement in 100mL of water within a 250mL beaker. Studies pharmacologically manipulating zebrafish behavior often deliver water soluble compounds via a bath solution within a beaker to be absorbed through the gills. Previous research in our lab has revealed that the beaker stress

paradigm (adapted from the bath solution method) reliably stimulates the release of cortisol, a physiological indicator of stress. We show here that this robust stress response is correlated with significant behavioral changes that have been observed within three well-utilized tasks in the literature, the novel tank dive, light-dark discrimination and open-field testing. A separate experiment sought to better understand this relationship as a function of time. Following the stressor, animals were placed in an aquatic open-field testing tank, and whole-body cortisol levels were subsequently measured over various time intervals during a 60 minute period. Mean cortisol levels were found to be highest during minutes 10 and 15 in the open field, and at minute 20, cortisol levels significantly decreased and remained relatively stable throughout minutes 25, 30, 45, and 60. All time points were also found to be significantly greater than baseline. Initial analysis revealed a strong correlation between cortisol levels and behavior change over time. Stress induced behavioral phenotypes, correlated with a measureable endocrine response, help to establish the zebrafish as a viable neurobehavioral model of stress and anxiety.

Disclosures: D.J. Echevarria: None. **A.D. Collier:** None. **D.J. Jouandot:** None. **C. Breazeale:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.01/GGG41

Topic: E.08. Biological Rhythms and Sleep

Support: NSF GRFP

Title: Neonicotinoid pesticide disrupts circadian locomotor behavior in *Drosophila*

Authors: *M. TACKENBERG[1], K. BROADIE[2], D. G. MCMAHON[2];
[1]Neurosci., Vanderbilt Univ., Nashville, TN; [2]Biosci., Vanderbilt, Nashville, TN

Abstract: A massive decrease in honeybee populations has been observed over the past ten years. This so-called Colony Collapse Disorder (CCD) has profound global implications, as honeybees may be responsible for the pollination of up to 80% of the United States' food producing crops, with a similar role worldwide. Studies over the past decade have identified a widely-used class of pesticides, nicotinic acetylcholine receptor agonists known as neonicotinoids, as a potential culprit for CCD. Further investigation into the effects of these pesticides on honeybees has revealed decreased forager return in bees given sublethal doses. We hypothesize that this change may be brought about through disruption of the circadian system of the bee, which it uses to navigate appropriately to and from hives. We have found that in

drosophila, sublethal exposure to the neonicotinoid thiamethoxam produces a disruption of circadian locomotor activity in LD cycles, with dosed flies exhibiting an increase in dark activity and corresponding decrease in light activity. Our results indicate that the pesticide may, in fact, be causing a disruption of the clock, which may lead to navigational issues in organisms that use circadian timekeeping for orientation.

Disclosures: M. Tackenberg: None. **K. Broadie:** None. **D.G. McMahon:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.02/GGG42

Topic: E.08. Biological Rhythms and Sleep

Title: Perinatal exposure to nicotine alters circadian system's response and synchronization in the juvenile mouse

Authors: *M. FUENTES-CANO[1], X. RINZA-FERNÁNDEZ[2], P. DURAN[1];
[1]Facultad De Ciencias, UNAM, Mexico City, Mexico; [2]Facultad De Ciencias, UNAM, Coyoacán, Mexico

Abstract: Tobacco and its main addictive component, nicotine have been associated to a large number of alterations in the development of the central nervous system (CNS) due to the interaction with the cholinergic system, in particular to the nicotine cholinergic receptors (nAChr). As is widely known, the cholinergic system (CS) and its nAChr are responsible for important processes during the CNS like development as neurogenesis. However, the study of the effects of nicotine during development in the circadian and system and particularly in the suprachiasmatic nuclei (SCN) remains an open question. The aim of this study was to analyze in juvenile mouse, the effects of perinatal exposure to nicotine on the circadian rhythm of locomotor activity as well as its physiological response to different photoperiods. In order to examine the SCN physiological integrity to maintain the circadian locomotor rhytms and its capacity to respond to resynchronization and constant light stress, ten 30 day-old male balb/c mouse per group, a control without treatment (Co) and a previously exposed to maternal 6mg/kg/day nicotine consumption, during gestation through lactation periods group (Pn), were submitted to a locomotor activity recording system (using infrared sensors). The recording period consisted in 65 consecutive days where the animals were exposed to: light/Dark (L:D) 12:12 by 5 days, constant darkness (CD) 0:24 by 25 days, L:D 12:12 by ten days and constant light (LC) 24:0 by 25 days. Data were analyzed using ACTIVIEW. Results showed a

consolidated circadian locomotor rhythm (CD tau=23.5 ±0.23hrs and CL tau=25.68±0.21 hrs) and resynchronization to 3±1 days in CL in the Co group. However, the Pn group increased their total locomotor activity but showed a deficient control circadian rhythm while in free running conditions (CD), since they became arrhythmic after 11 ±3 days in CD, and failed to resynchronize to LD (5±1 days). The aforementioned results suggest that the cholinergic system altered by perinatal nicotine exposure, in particular its nAChr, had a direct influence on the SCN and/or the whole circadian system, probably altering the photic response to external stimuli in the mid and long term. We conclude that the cholinergic system overstimulation (throughout the nicotinic receptors) during the critical periods of perinatal development may affect the circadian system maduration and the locomotor activity rhythm as well as the photic stimuli synchronization. Finally, it is possible that the nicotinic receptor expression and the cholinergic regulation alters the circadian system neuroanatomical substrate during those developmental critical periods.

Disclosures: M. Fuentes-Cano: None. **X. Rinza-Fernández:** None. **P. Duran:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.03/GGG43

Topic: E.08. Biological Rhythms and Sleep

Support: Swedish Research Council (VR) Grant 21379

Title: Analysis of complexity and regularity of spontaneous locomotion in group-housed mice: A novel predictive approach based on freely behaving animals

Authors: *S. SPULBER, M. CONTI, N. ONISHCHENKO, S. CECCATELLI;
Karolinska Institutet, Dept. of Neurosci., Stockholm, Sweden

Abstract: Exploration is a purposeful activity, defined as behavioural acts and postures that allow the collection of information about objects or parts of the environment. Spontaneous locomotor activity in a familiar environment displays cyclic fluctuations in relation to environmental, social stimuli and internal physiological processes. The aim of this study was (1) to investigate the complexity of spontaneous locomotion in group-housed mice; (2) to characterize the changes in response to the suppression of the circadian dark-light cycle; and (3) to identify possible correlates in behavioural disturbances assessed by classical behavioural tests. Adult male C57Bl/6 mice were implanted with subcutaneous radio frequency identification (RFID) tags. We used the TraffiCage™ system, which consists of an array of antennas placed

under the cage that detect the RFID tags, and allows the long-term monitoring of locomotor activity in group-housed mice. The raw data were exported and analysed offline using custom implementations of algorithms for assessing the underlying regularity (approximate entropy, ApEn), the fractal-like structure (analysis of scaling behaviour by means of detrended fluctuation analysis, DFA), and the coefficient of determination for spontaneous locomotion in the homecage. We found that spontaneous locomotion in group-housed adult mice displays a scale-invariant complexity (fractal-like) with a scaling exponent similar to the one measured in rats and humans, and displays a strong underlying regularity. The complexity is decreasing, while the regularity and the coefficient of determination increase when the dark-light cycle is suppressed for extended periods. To validate the interpretation of the above-mentioned alterations, we used genetically modified and pharmacologically induced models (FMR1-KO; prenatal exposure to the synthetic glucocorticoid dexamethasone; acute neuroinflammation induced by systemic administration of LPS). In each model we found specific alterations in the pattern of spontaneous locomotion in the homecage that were consistent with the findings from classical behaviour tests, including open field and hole board exploration, and social recognition. The identified set of parameters derived from the pattern of spontaneous locomotion in the homecage of group-housed mice, displayed early specific changes in the different experimental models, which appeared to be predictive of particular behavioural alterations. The possible correlation of gene expression patterns with alterations in locomotor activity is under investigation.

Disclosures: S. Spulber: None. **M. Conti:** None. **N. Onishchenko:** None. **S. Ceccatelli:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.04/GGG44

Topic: E.08. Biological Rhythms and Sleep

Title: Involvement of tPA and LRP-1 in regulating mammalian circadian clock phase

Authors: *J. COOPER, R. A. PROSSER;
Biochem. and Cell. and Mol. Biol., Univ. of Tennessee, Knoxville, TN

Abstract: The suprachiasmatic nucleus (SCN) contains the primary mammalian circadian clock. Photic entrainment of the clock depends on glutamate release from retinal ganglion cells onto SCN neurons. *In vitro* glutamate delays circadian clock phase when applied to the SCN in the early night, and advances phase in the late night. Glutamate-induced phase shifts require concurrent activation of TrkB receptors by brain-derived neurotrophic factor (BDNF). We

previously showed that tissue-type plasminogen activator (tPA) proteolytic activity regulates glutamate-induced phase shifts *in vitro*: tPA cleaves plasminogen into plasmin; plasmin cleaves pro-BDNF into its active form m(ature) BDNF; and mBDNF binds TrkB receptors allowing clock phase shifts (Mou et al 2009). In addition to its proteolytic activity, tPA elicits non-proteolytic effects (such as endocytosis and/or activation of signaling pathways) by binding various receptors, one of which is lipoprotein receptor-related protein 1 (LRP-1). In this study we investigate the effects of inhibiting LRP-1 on glutamate induced phase shifts, using either receptor associated protein (RAP) or an anti-LRP-1 polyclonal antibody as inhibitors of LRP-1 activity. SCN brain slices from adult male C57BL/J6 mice were treated with glutamate (1mM) +/- RAP (500nM) or anti-LRP-1 antibody (75µg/mL) at ZT 16 or ZT 23 (where ZT 0=lights-on in the animal colony) for 10 minutes. The following day we recorded SCN single-unit neuronal activity (SUA) to determine the time of peak activity. The results indicate that concurrent application of RAP or anti-LRP-1 with glutamate prevents the normal shifts, while RAP alone has no effect on clock phase. These results suggest that in the SCN LRP-1 activity is important for glutamate-dependent clock phase regulation. tPA and LRP-1 may represent converging pathways, or may be independent of each other. We are investigating the interrelationship of these two pathways using tPA knockout (KO) mice, B6.129S2-Plat^{tm1Mlg}/J. First we characterized the SUA rhythm in control tPA KO brain slices and after glutamate treatment. SCN slices from adult male tPA KO mice exhibit entrained neuronal activity rhythms, and 10µM glutamate at ZT16 and ZT23 induces phase delays and phase advances, respectively. Thus, tPA KO mice do not exhibit severe deficiencies in clock phase regulation, possibly reflecting redundant mBDNF-generating pathways. Finally, preliminary data suggest that RAP does not inhibit glutamate phase resetting in tPA KO slices, indicating LRP-1 may work through a tPA-dependent mechanism. Future studies will continue exploring tPA and LRP-1 regulation of SCN circadian clock phase.

Disclosures: J. Cooper: None. **R.A. Prosser:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.05/GGG45

Topic: E.08. Biological Rhythms and Sleep

Support: Donald Akers Fellowship

Title: Matrix metalloproteinases 2/9 as potential regulators of the suprachiasmatic nucleus circadian clock

Authors: K. E. ABRAHAMSSON, *R. A. PROSSER;
Dept Biochem, Cell & Mol Biol, Univ. Tennessee, KNOXVILLE, TN

Abstract: A multitude of organisms exhibit rhythmic behavioral and metabolic processes that align with light and dark cycling of the environment. This phenomenon, called photic entrainment, is regulated in mammals by a collection of neurons in the suprachiasmatic nucleus (SCN) that comprise the master circadian clock. As abnormal entrainment of mammalian circadian rhythms is linked to sleep and behavioral disorders, clarifying the molecular mechanisms that regulate SCN clock phase is significant. It is known that during the subjective night, exposure to light or *in vitro* application of glutamate causes a delay in peak neuronal activity. In addition to the complex intracellular processes critical to photic phase resetting, a variety of extracellular proteins are known to regulate photic/glutamate phase-shifting processes within the SCN. One example is the conversion of pro-Brain Derived Neurotrophic Factor (pBDNF) to its mature form (mBDNF), allowing it to activate TrkB receptors, a necessary step for glutamate- and light-induced phase shifts. While our previous work has implicated tissue-type plasminogen activator (tPA) in this proteolytic step, in the hippocampus, the conversion of pBDNF to mBDNF is catalyzed by the matrix metalloproteinases MMP2 /MMP9. MMP2/MMP9 are classically known to serve as extracellular matrix remodelers, but our work aims to investigate MMP2/MMP9 involvement in SCN clock phase regulation. For electrophysiology experiments, we used acute SCN brain slices prepared from adult, male C57BL/6 mice. Slices were maintained for two days *in vitro* and had bath-application of drugs to the slices at Zeitgeber time (ZT) 16 (where ZT 0=lights on and ZT 12=lights-off in the animal colony) on the first day. We recorded the spontaneous activity of SCN neurons using extracellular electrodes the day following application of drug treatment. We found that *in vitro* application of the MMP2/MMP9 inhibitor ((2R)-[(4-Biphenylylsulfonyl)amino]-N-hydroxy-3-phenylpropionamide or BiPS; 100nM-10uM) alone for 50 min at ZT 16 induced 2-3 h delays, similar to the effects of glutamate. This result was contrary to our initial hypothesis and led us to think that MMP2 and/or MMP9 are acting in the SCN to regulate NMDA receptor signaling rather than through the conversion of pBDNF to mBDNF. To test this, we are currently examining whether BiPS induces phase advances when applied at ZT 23, and whether the phase shifts induced by BiPS are blocked by an NMDA receptor antagonist [(2 R)-amino-5-phosphonopentanoate; AP5]. Combining the aforementioned studies with temporal protein activity and expression assays will reveal the role of MMP2/MMP9 in the SCN.

Disclosures: K.E. Abrahamsson: None. **R.A. Prosser:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.06/GGG46

Topic: E.08. Biological Rhythms and Sleep

Support: IOS-1051919

08-TMH070343B

Title: Effects of intergeniculate leaflet (IGL) lesions on behavioral and brain responses to photic stimuli in diurnal grass rats

Authors: *A. J. GALL, L. SMALE, L. YAN, A. NUNEZ;
Michigan State Univ., East Lansing, MI

Abstract: The intergeniculate leaflet (IGL), a subdivision of the lateral geniculate complex, receives direct retinal input, exhibits light-induced Fos expression, and is involved in masking in both nocturnal and diurnal species (Moore & Card, 1994; Prichard et al., 2002; Redlin et al., 1999; Gall et al., 2013). We have recently shown that IGL lesions cause diurnal grass rats to decrease their activity in response to a light pulse given at Zeitgeber time (ZT) 14; in control grass rats, a light pulse increases general activity at this time. The objective of the current study was to use Fos to shed light on pathways through which the IGL might influence this masking effect of light in grass rats. To do this, we examined the effects of IGL lesions on the photic response in two retinorecipient brain areas, the suprachiasmatic nucleus (SCN) and the olivary pretectal nucleus (OPT). The SCN and OPT are light responsive and connect reciprocally with the IGL. Because we previously found that the period of activity rhythms was unaffected by IGL lesions in both constant darkness (DD) and constant light (LL), we predicted that the Fos response to light in the SCN would be similar for both groups, whereas downstream regions from the IGL (such as the OPT) would exhibit a change in Fos expression. Here, 1-h light pulses were presented from ZT14 to ZT15 in IGL lesioned and sham operated grass rats. Animals were either sacrificed at ZT15 at the end of the light pulse, or sacrificed at the same time without receiving the light pulse. We found that following the light pulse, both shams and animals with IGL lesions exhibited a significant increase in Fos expression in the SCN. In contrast, Fos expression in the OPT was significantly increased in shams following a light pulse, whereas in animals with IGL lesions, Fos expression in the OPT was significantly decreased following the light pulse. Altogether, our results suggest that the OPT, but not the SCN, changes its responses to light following IGL lesions that reverse masking responses in diurnal grass rats. These data raise the possibility that interconnections between the IGL and OPT may play a role in determining the direction of the behavioral response to light at this time.

Disclosures: A.J. Gall: None. **L. Smale:** None. **L. Yan:** None. **A. Nunez:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.07/GGG47

Topic: E.08. Biological Rhythms and Sleep

Title: Investigating MAPK pathway involvement in TTM- and Cu-induced phase shifts of the SCN circadian clock

Authors: *Y. YAMADA, R. A. PROSSER;
Biochemistry, Cell. and Mol. Biol., Univ. of Tennessee, Knoxville, TN

Abstract: The suprachiasmatic nucleus (SCN) is the mammalian master clock coordinating circadian rhythms under internal and environmental regulation. Light stimulates specialized retinal cells to release glutamate (Glu) onto circadian clock neurons of the SCN. During the subjective night, Glu activation of NMDA receptors (NMDAR) shifts clock phase by activating Ca^{2+}-dependent signaling pathways and downstream gene expression. One such pathway is the activation of MAPK/ ERK. Using acute brain slices containing the SCN prepared from adult, male C57Bl/J6 mice, we have shown that a copper (Cu)-specific chelator, tetrathiomolybdate (TTM), causes phase shifts in SCN neuronal activity rhythms when bath-applied for 10 min. Although TTM-induced phase shifts at Zeitgeber time (ZT) 16 and 23 (where ZT 0 = lights-on and ZT 12 = lights-off in the donor animal colony) are NMDAR-dependent, TTM also induces daytime phase shifts when applied at ZT 6 that are independent of NMDAR activation. Here we have investigated the mechanisms through which Cu modulates SCN clock phase. Previous studies show that Cu application increases MAPK/ERK signaling in various cell types, including cerebellar granule neurons. Furthermore, one study (Molecular and Cellular Biology (2012): 32, 1284-1295) demonstrated that Cu-induced ERK activation is: reduced by Cu chelators TTM or bathocuproine disulfonate; induced upon Cu binding to MEK1 (a MAPK kinase) *in vitro*; and dependent on Cu import by Copper transporter-1 (CTR1) *in vivo*. Based on these findings, we first used immunoblot procedures to show that CTR1 protein is expressed in the SCN. Next, using our SCN brain slice preparation, we determined that TTM is still able to induce *in vitro* phase delays when it is co-applied with the MEK inhibitor U0126 at ZT16, while U0126 attenuates Glu-induced phase delays *in vitro*. These results suggest that TTM-induced phase delays do not require MEK1 activity, but instead involve an alternative, intracellular mechanism downstream of NMDAR activation. On-going experiments at ZT6 and ZT23 with U0126 will help elucidate the cellular mechanisms through which TTM modulates SCN circadian clock phase at these other times. Along other lines, we have also shown that *in vitro* Cu application causes similar phase shifts during the subjective night as those induced by Cu chelators. Therefore, in future experiments we will test whether MEK inhibition blocks these Cu-induced phase shifts. Overall, the role of Cu in the SCN remains unclear, but the putative interaction between Cu homeostasis and circadian rhythms highlights the need for further investigation.

Disclosures: Y. Yamada: None. **R.A. Prosser:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.08/GGG48

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant AA017898

Title: Alcohol tolerance and withdrawal using the suprachiasmatic nucleus (SCN) circadian clock as a model system

Authors: *J. H. LINDSAY[1], J. D. GLASS[2], R. A. PROSSER[3];
[1]BCMB, Univ. of Tennessee Knoxville, Knoxville, TN; [2]Kent State Univ., Kent, OH; [3]Univ. of Tennessee, Knoxville, TN

Abstract: Alcohol abuse is linked to cognitive defects, depression, and sleep disturbances. The strong connection between sleep and circadian disruptions led us to investigate ethanol's effect on the circadian clock. We have shown that acute ethanol blocks photic phase shifts *in vivo* and glutamatergic phase shifts *in vitro*. Studies have shown that ethanol consumption often leads to acute, rapid and/or chronic tolerance. We have demonstrated that the SCN clock exhibits acute (developing in <30 min) tolerance to ethanol *in vitro*. We are currently investigating rapid (develops in 8-24 h) tolerance to ethanol. For these experiments C57BL/6 mice were given access to 15% ethanol during a single night. SCN brain slices were made the following morning and treated at either zeitgeber time (ZT) 16 (where ZT 0 = lights-on and ZT 12 = lights off) or ZT 23 with glutamate (1mM) +/- ethanol for 10 min. The next day spontaneous SCN neuronal activity was monitored across the 12 h subjective day period to determine the time of peak activity. 20mM ethanol blocks glutamatergic phase shifts in brain slices from ethanol-naïve mice, while 200mM ethanol is needed to induce the same effect in tissue from ethanol-exposed mice. Surprisingly, this tolerance lasts 48-96 hr after ethanol consumption. We are also investigating the effects of chronic ethanol consumption on SCN clock phase resetting, where mice are given access to free choice 15% ethanol or water *ad lib* for 10-14 days. Our data thus far indicate that chronic tolerance develops to *in vitro* glutamate-induced phase shifts, with a similar shift in the effective concentration of ethanol as seen in the rapid tolerance paradigm. Finally, we are investigating the response to ethanol withdrawal in our *in vitro* SCN brain slice preparation. For these experiments adult C57 mice have access to 15% ethanol for three consecutive weeks under a drinking in the dark paradigm. SCN brain slices are then prepared

after a 2 day ethanol withdrawal period. Preliminary results suggest that under these conditions the SCN clock exhibits an increased sensitivity to phase-resetting to glutamate. Future experiments will expand on these studies, including investigating changes in protein expression/phosphorylation in the SCN.

Disclosures: J.H. Lindsay: None. **J.D. Glass:** None. **R.A. Prosser:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.09/HHH1

Topic: E.08. Biological Rhythms and Sleep

Support: The Royal Society

Title: K2P channels play a key role in defining irradiance detection and circadian photoentrainment in mice

Authors: *L. A. ATKINSON, A. A. MATHIE, G. S. LALL;
Univ. of Kent, Chatham, Kent, United Kingdom

Abstract: In mammals, the planetary day-night cycle acts as the principal signal for daily alignment of the biological circadian clock to external time. This synchronisation of behavioural and physiological rhythms is achieved through regulation of peripheral clocks in all major organ systems, choreographed by a central pacemaker within the hypothalamus known as the suprachiasmatic nucleus (SCN).
Light enters the biological system exclusively via the mammalian eye, where the retina utilises three major classes of photoreceptor, rods, cones and melanopsin-containing retinal ganglion cells to decode environmental illuminance for non-image forming (NIF) responses of circadian photoentrainment and the pupillary light reflex. Decoding of light information at the level of the retina and SCN communication to the periphery both rely on neuronal excitation mechanisms, which in the circadian system, are achieved through changes in neuronal membrane potential, from a resting potential stabilised by two-pore domain potassium leak channels (K2P channels). In the present study, using a transgenic mouse model with a deletion of a specific K2P channel, we investigate the role of K2P channels in regulating NIF responses to light from initial retinal decoding to behavioural output of the clock. Through locomotor activity studies and pupillometry we show attenuation in photoentrainment ability and alterations in pupillary light reflex in the transgenic model compared to wild-type equivalents, demonstrating the fundamental role of K2P channels in the regulation of circadian rhythms. Additionally, through the use of

specific-wavelength monochromatic light, we are able to attribute contributions of individual classes of photoreceptor to NIF responses to light.

Disclosures: L.A. Atkinson: None. **G.S. Lall:** None. **A.A. Mathie:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.10/HHH2

Topic: E.08. Biological Rhythms and Sleep

Title: Dim light at night does not disrupt timing or quality of sleep in mice

Authors: *J. C. BORNIGER, Z. M. WEIL, N. ZHANG, R. J. NELSON;
Neurosci., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Artificial nighttime illumination has recently become commonplace throughout the world, however, in common with other animals, humans have not evolved in the ecological context of chronic light at night. With prevailing evidence linking the circadian, endocrine, immune, and metabolic systems, understanding these relationships is important to understand the etiology and progression of several diseases. To eliminate the covariate of sleep disruption in light at night studies, researchers often use nocturnal animals. However, the assumption that light at night does not affect sleep in nocturnal animals remains unspecified. To test the effects of light at night on sleep, we maintained Swiss-Webster mice in standard light/dark (LD) or dim light at night (DLAN) conditions for 8-10 weeks and then measured EEG and EMG biopotentials via wireless telemetry over the course of two consecutive days to determine differences in sleep timing and homeostasis. Results show no statistical differences in total percent time, number of episodes, maximum or average episode durations in wake, slow-wave sleep (SWS), or rapid-eye movement (REM) sleep. No differences were evident in SWS delta power, an index of sleep drive, between groups. Mice kept in DLAN conditions showed a relative increase in REM sleep during the first few hours after the dark-light transition. Both groups displayed normal 24 h circadian rhythms as measured by voluntary running wheel activity. Groups did not differ in body mass, but a marked negative correlation of body mass with percent time spent awake and a positive correlation of body mass with time spent in SWS was evident. Elevated body mass was also associated with shorter maximum wake episode durations, indicating heavier animals had more trouble remaining in the wake vigilance state for extended periods of time. Body mass did not correlate with activity levels, nor did activity levels correlate with time spent in different sleep states. These data indicate that heavier animals tend to sleep more, potentially contributing

to further weight gain. We conclude that chronic DLAN exposure does not significantly affect sleep timing or homeostasis in mice, supporting the use of dim light with nocturnal rodents in chronobiology research to eliminate the possible covariate of sleep disruption.

Disclosures: J.C. Borniger: None. **Z.M. Weil:** None. **N. Zhang:** None. **R.J. Nelson:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

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Program#/Poster#: 281.11/HHH3

Topic: E.08. Biological Rhythms and Sleep

Support: NICHD36460

Title: Rapid circadian reentrainment after simulated travel to anti-meridian time zones in Syrian hamsters

Authors: *E. M. HARRISON, M. R. GORMAN;
UCSD, La Jolla, CA

Abstract: Circadian adjustment after travel across time zones is frustratingly slow in humans and other mammals, occurring at the rate of approximately an hour a day. It has recently been established in rodent models, however, that re-entrainment can be markedly accelerated via circadian waveform manipulation induced by changes in the light/dark schedule. In particular, bifurcation of circadian waveform under permissive 24 h light:dark:light:dark (LDLD) cycles results in an unprecedented acceleration of entrainment to a new schedule. Recently, our lab has found that Syrian hamsters in a bifurcated state for 14 days adjust wheel-running activity almost immediately after transfer to any of 6 equidistant LD schedules four hours apart. To determine whether this rapid adjustment of the activity rhythm in LD is matched by a shift in the endogenous circadian pacemaker, hamsters bifurcated under a 8:4:8:4 light:dark schedule were challenged to phase-shift to one of two time-zones 12 h apart for 3 cycles and subsequently released into constant darkness (DD). Analysis of free-running rhythms indicated that the pacemaker was entrained such that night onset occurred within 1.5 h of the new scotophase, indicating entrainment to schedules 12 h in anti-phase within days. Further, to determine the minimum number of days of bifurcation required to achieve this enhanced rate of resetting, 48 hamsters were bifurcated for a period of 3, 5, 7 or 14 consecutive days (n= 8 in each condition) before being moved to one of two LD 16:8 conditions as described above. Preliminary data suggest as little as a week or less may be required for enhanced resetting. Experiments will determine whether fewer days of bifurcation are sufficient to enhance resetting.

Disclosures: E.M. Harrison: None. **M.R. Gorman:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.12/HHH4

Topic: E.08. Biological Rhythms and Sleep

Title: Alterations in light signalling are key contributors to the decline of circadian photoentrainment in aged mammals

Authors: S. M. BIELLO[1], D. R. BONSALL[2], L. A. ATKINSON[3], P. C. MOLYNEUX[2], M. E. HARRINGTON[2], *G. S. LALL[3];
[1]Psychology, Univ. of Glasgow, Glasgow, United Kingdom; [2]Neurosci., Smith Col., Northampton, MA; [3]Medway Sch. Pharm., Univ. of Kent, Kent, United Kingdom

Abstract: Daily changes in the environmental light-dark cycle provide the fundamental synchronising cue for physiological and behavioural circadian entrainment. The suprachiasmatic nucleus (SCN), located in the brain, integrates both the environmental irradiance and behavioural/ social stimuli to output a synchronising signal to which global physiological systems entrain, and as such, defines the SCN as the master circadian pacemaker. NMDA driven signalling pathways mediate the majority of light responses at the level of the SCN. Recently, the age related decline in circadian entrainment has been correlated to alternations in key SCN cellular mechanism, ultimately impacting output synchronisation signals.
In the current study, we investigate changes in light driven SCN resetting in aged mice (C57BL/6) at both behavioural and cellular levels. Behavioural circadian rhythms were measured using daily wheel running activity recordings. Old mice (>18 months) showed decreased phase resetting to light at both dim (30 lux) and bright (300 lux) light. In order to ascertain if this effect was attributed to a NMDA driven pathway at the level of the SCN we utilised pharmacological and molecular techniques to isolate this particular receptor class. *In vivo* microinjections of NMDA directly into the third ventricle resulted in significantly less behavioural resetting in aged mice relative to younger (6 months) counterparts. Real-time PCR analysis of the relative expression of specific NMDA receptor subunits revealed a significant reduction in the NR2B subunit. The functional impact of this finding was confirmed using a specific NR2B antagonist, *in vivo*, which resulted in attenuating light phase resetting in the young; an effect that was absent in the aged experimental group.
In conclusion, our results demonstrate that the age related decline in circadian synchronisation is, in part, attributed to the ability of the SCN in interpreting light information through the NMDA

pathway. More specifically this can be narrowed down to changes in specific NMDA subunits, in particular the NR2B subunit.

Disclosures: S.M. Biello: None. **D.R. Bonsall:** None. **L.A. Atkinson:** None. **P.C. Molyneux:** None. **M.E. Harrington:** None. **G.S. Lall:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.13/HHH5

Topic: E.08. Biological Rhythms and Sleep

Support: ONR N000141310285

Title: Light intensity requirements for induction and maintenance of bifurcated circadian rhythms in mice under light:dark:light:dark cycles

Authors: *J. SUN[1], M. GORMAN[2];
[1]Psychology 0109, Gorman Circadian Res. Lab., La Jolla, CA; [2]Psychology 0109, Univ. of California - San Diego, La Jolla, CA

Abstract: The mammalian circadian pacemaker, which controls daily rhythms in physiology and behavior, is comprised of multiple oscillators that under a variety of natural and unnatural conditions may be temporally dissociated. In a recently described rodent model system, herein termed bifurcation, exposure to a 24 h light:dark:light:dark cycle induces the circadian pacemakers of rodents generate two subjective days and two subjective nights per 24 hour cycle. Bifurcation of the circadian pacemaker markedly increases adjustment after phase shifts, and has been proposed as a potential solution for night-shift workers due to its flexiblity, robustness, and stability. Mechanistically, bifurcation reflects a change in the coupling between multiple oscillators herein referred to as day and night oscillators. These two oscillators are maintained in a near anti-phase relationship via the interposition of bright light in between each bout of activity as suggested when bifurcated animals rejoin readily when subjected to constant darkness. Prior bifurcation has been achieved through the use of dim scotopic illumination (DSI) (<0.1 lux) in conjunction with bright (>100 lux) photophasic illumination and the introduction of a bifurcated light schedule as DSI is believed to reduce the coupling forces promoting rejoining. Except for the fact that DSI facilitates induction of bifurcation, little else is known about the properties of the environmental light cycle that induce and maintain bifurcation, especially that of photophasic light. The current study seeks to determine how timing of DSI affects bifurcation and the threshold of bright light required to keep animals entrained to a bifurcated light schedule. Prior to

manipulations, C57/Bl6J mice were bifurcated (7:5:7:5 LDLD) with DSI in both scotophases. After two weeks, mice were randomly assigned to one of two groups where DSI was subsequently dropped from either the day or night scotophase. Removal of DSI in either scotophase did not affect bifurcated entrainment. Bifurcation was maintained uniformly after gradual reduction of photophasic light intensity every 2 weeks from >100 lux to 1.0 lux regardless of the scotopic placement of DSI. Likewise, bifurcation was induced in unbifurcated mice transferred to a bifurcated 7:5:7:5 LDLD schedule with photophasic light intensities of 1.0 lux and DSI in either day or night scotophase. We show a) that timing of DSI does not affect the maintenance of bifurcated rhythms and b) that very little photophasic light is required to induce or maintain bifurcation. Thus, bifurcation may be induced and maintained in a greater variety and with lower light intensities than previously described.

Disclosures: J. Sun: None. **M. Gorman:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

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Topic: E.08. Biological Rhythms and Sleep

Support: Finnish Graduate School in Neurosciences

Academy of Finland

Title: Changes in sleep architecture of C57BL/6J mice in response to a short photoperiod

Authors: *S. V. ROZOV[1], J. C. ZANT[2], T. H. PORKKA-HEISKANEN[2], P. PANULA[1]; [1]Anat., [2]Physiol., Inst. of Biomedicine, Helsinki, Finland

Abstract: The ways the circadian system affects the sleep-wake cycle can be assessed by several means. One of these, known as forced desynchrony, has been successfully applied in human and rat studies.It induces the uncoupling of the diurnal rhythmicity of sleep-wake behaviour, melatonin synthesis and other rhythms into an endogenously driven circadian, and the light-entrained component enabling their independent examination. We aimed to implement this protocol on mice as a model system widely used in sleep and circadian research. C57BL/6J mice were implanted with EEG/EMG electrodes and after a one week recovery period comected to a CED1401 data acquisition unit (Cambridge Electronic Design). EEG were recorder for two weeks during a normal light-dark cycle, after which the mice were to subjected to symmetric light-dark cycles of decreasing lengths: 24→22→21→20 h (170 lux light phase, <0.5 lux dark

phase; each period lasing 2 weeks). Behavioural activity of the animals showed no signs of rhythm splitting; instead it was completely entrained to all periods mentioned above. It was accompanied by a sharp progressive decrease in total locomotion although the overall % of time spent in wakefulness, NREM or REM sleep remained unchanged between different period lengths, as was shown by EEG analysis. In contrast to previous reports on rat where shortening the rhythm leads to dissociation of circadian and entrained rhythmic components, the mice in our study demonstrated a significant progressive rearrangement of the sleep-wake architecture towards an even distribution of sleep and wake amounts over the light-dark cycle. Altogether this data suggests potential differences between rats and mice in their circadian modulation of sleep and entrainment properties of the circadian oscillator.

Disclosures: S.V. Rozov: None. **J.C. Zant:** None. **T.H. Porkka-Heiskanen:** None. **P. Panula:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.15/HHH7

Topic: E.08. Biological Rhythms and Sleep

Support: NSERC

Title: The effect of chronic photoperiod shifting on metabolism and stress in female Long-Evans rats

Authors: *S. DEIBEL, S. M. DERKSEN, N. S. HONG, R. J. MCDONALD;
Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Shift work can have detrimental effects on the brain and body. Shift workers have a higher prevalence of diseases, such as, dementia, cardiovascular disorders, cancer, metabolic syndrome, and obesity. Circadian disruption is a possible mechanism for these effects, as shift workers circadian rhythms are in a continuous state of desynchrony because they are not able to fully entrain to the aberrant light-dark cycles. While circadian disruption results in cognitive impairments, the effects on physiological processes such as metabolism and the stress response are less clear. The current set of experiments assessed the effects of chronic circadian disruption on stress and metabolism in female Long-Evans rats. A 64-day chronic photoperiod shifting paradigm was used to disrupt circadian rhythms. During photoperiod shifting the animals had access to high and low fat diets. Consumption rates of both diets were measured throughout the duration of the experiment. To determine if stress was elevated during chronic photoperiod

shifting, serum corticosterone values were quantified for each of the four cycles of phase shifting. Additionally, following photoperiod shifting serum glucose values were measured during fasting and, after a glucose challenge test. Surprisingly, the shifted animals did not gain more weight or exhibit a preference for either of the diets during photoperiod shifting. However, glucose values during fasting and, in response to a glucose challenge test were elevated in the photoperiod shifted rats. This study demonstrates that chronic photoperiod shifting has deleterious effects on metabolism, and the ability to cope with stress in the same individual.

Disclosures: S. Deibel: None. **S.M. Derksen:** None. **N.S. Hong:** None. **R.J. McDonald:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

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Topic: E.08. Biological Rhythms and Sleep

Support: Hong Kong Theme-based Research Scheme

Title: Melatonin does not improve re-entrainment after light-dark cycle shift in mice with the double knockout of Epac1 and Epac2

Authors: *Z. XIAN[1], S. CHEUNG[3], Y. WONG[3], S. CHUNG[1,2];
[2]Res. Ctr. of Heart, Brain, Hormone & Healthy Aging, [1]The Univ. of Hong Kong, Hong Kong, Hong Kong; [3]Div. of Life Sci., The Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong

Abstract: Exchange protein directly activated by cAMP (Epac), which consists of two isoforms, Epac1 and Epac2, is a downstream effector of cAMP independent of protein kinase A. Previously, a non-selective Epac analog was used to establish its involvement in the maintenance of circadian rhythm. In order to determine which isoform of Epac is involved in the regulation of circadian rhythm, mice of wild-type ($Epac^{+/+}$), homozygous knockout of Epac1 ($Epac1^{-/-}$), homozygous knockout of Epac2 ($Epac2^{-/-}$) and homozygous knockout of Epac1 and Epac2 ($Epac1^{-/-}$; $Epac2^{-/-}$) were subjected to re-entrainment experiments, which the onset of the dark cycle was advanced by 6 hours. $Epac1^{-/-}$; $Epac2^{-/-}$ mice required a longer time to re-entrain to the advance of the dark cycle than that of WT mice. No significant difference in re-entrainment was observed among $Epac^{+/+}$, $Epac1^{-/-}$ and $Epac2^{-/-}$ mice, suggesting that Epac1 and Epac2 might compensate in the absence of a single isoform of Epac. The delay in re-entrainment in $Epac1^{-/-}$; $Epac2^{-/-}$ mice might be due to altered *Clock* gene expression in hypothalamic suprachiasmatic nucleus (SCN), which is the principle pacemaker of circadian rhythm. It has been reported that

Clock mutant mice have a higher sensitivity to light stimulus with lower rhythmic expression of circadian genes *Per1* and *Per2*. Quantitative real-time PCR analysis was performed to determine the *Clock* gene expression in the SCN of the four genotypes of mice. Epac1$^{-/-}$; Epac2$^{-/-}$ mice showed a higher mRNA expression of *Clock* gene in the SCN compared that of Epac$^{+/+}$, which possibly contributed to the delayed re-entrainment in the Epac1$^{-/-}$; Epac2$^{-/-}$ mice. Interestingly, melatonin injection (4 mg/kg in 3% ethanol/saline, *s.c.*) at 30 min before the post-shift dark onset for 3 consecutive days effectively accelerated the re-entrainment in Epac$^{+/+}$ mice, whereas Epac1$^{-/-}$; Epac2$^{-/-}$ mice did not improve in re-entrainment following melatonin injection, suggesting that melatonin mediates phase shift of circadian rhythm via the cAMP-Epac pathway. Similarly, same dose of melatonin injection did not facilitate the re-entrainment in melatonin receptor MT_1 KO mice, whereas MT_2 KO mice were responsive to melatonin treatment. The similar phenotypes between Epac1$^{-/-}$; Epac2$^{-/-}$ mice and MT_1 KO mice suggest that melatonin facilitates the re-entrainment by activating MT_1 receptor and Epac.

Disclosures: Z. Xian: None. **S. Cheung:** None. **Y. Wong:** None. **S. Chung:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

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Topic: E.08. Biological Rhythms and Sleep

Support: DGAPA, PAPIIT IN225311

DGAPA. Postdoctoral support to D. Luna-Moreno

Title: Obese mice Neotomodon alstonii present an altered phase in daily rhythms of hypothalamic leptin and ghrelin receptors

Authors: D. LUNA-MORENO[1], *M. MIRANDA-ANAYA[1], C. LUNA-ILLADES[1], A. CARMONA-CASTRO[2];
[1]Unidad Multidisciplinaria de Docencia e Investigación, Facultad De Ciencias, UNAM, Juriquilla Querétaro, Mexico; [2]Facultad De Ciencias, UNAM, México D.F., Mexico

Abstract: Obesity affects a wide and increasing population in industrialized world. For the past several years, various approaches have been used to identify the physiological and genetic bases of obesity; among the most studied are the mechanisms regulating food intake, mediated by hormones and neurotransmitters controlling the sensations of satiety and hunger in specific centers within the hypothalamus. Recent findings indicate that exist a narrow relationship

between circadian deregulation and obesity, also, obesity itself may increase circadian internal uncoupling of circadian oscillators.
The present work was aim to compare differences in hypothalamic receptors of leptin (LR) and ghrelin (GHS-R), from brains collected along the light-dark cycle in lean and obese female mice Neotomodon alstoni, a mouse that spontaneously develops overweight and obesity when fed with a regular diet for laboratory rodents, specially the females. Obese mice show altered circadian rhythmicity as well as parameters related with the metabolic syndrome.
Lean (n=20, 45± 2g) and obese (n=15, 70± 4g) mice 6-8 months old were collected during the diestrous phase, at different hours in a LD cycle (0,5,10,15,19 h). Brains were frozen and hypothalamus was separated, then the homogenate was protein extracted for Western Blot analysis. A different group of animals (7 lean and 7 obese) were used to monitor changes in locomotor activity in a freely moving cage, with infrared sensors and feeding ad libitum.
Our results indicate that obese mice display less activity during the night, but are more active than lean during the day. Also, obese mice eat more than lean. Western blot analysis of hypothalamic receptors indicates that the daily rhythms of LR and GHS-R present different acrophase in obese mice Neotomodon alstoni. The aforementioned indicates that overfeeding in Neotomodon may be related with a delayed hunger signal in hypothalamus of obese together with an insufficient feedback of the leptin system.

Disclosures: D. Luna-Moreno: None. **M. Miranda-Anaya:** None. **C. Luna-Illades:** None. **A. Carmona-Castro:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

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Topic: E.08. Biological Rhythms and Sleep

Support: NSF Grant DMS-1121361

Title: Modeling spontaneous internal desynchrony of sleep-wake behavior and the circadian rhythm in humans

Authors: V. BOOTH[1], R. GLEIT[1], *C. DINIZ BEHN[2];
[1]Mathematics, Univ. of Michigan, Ann Arbor, MI; [2]Mathematics, Gettysburg Col., Gettysburg, PA

Abstract: When entrained to daily environmental time cues, human sleep-wake behavior cycles with an approximately 24 h period in phase with the circadian rhythm coordinated by the

suprachiasmatic nucleus (SCN). When isolated from external environmental time cues for extended periods of time, sleep-wake behavior can spontaneously desynchronize from the circadian rhythm and the two cycles no longer display the same period or a constant phase relationship. Usually, the circadian rhythm period remains close to 24 h while the sleep-wake cycle period has been reported to either increase or decrease. We used a physiologically based mathematical model of a sleep-wake regulatory network to replicate typical human sleep patterns and spontaneous internal desynchrony behavior as observed under temporal isolation conditions. The model sleep-wake regulatory network described the neurotransmitter mediated interactions among brainstem and hypothalamic neuronal populations that participate in the transitions between wake, rapid eye movement (REM) sleep and non-REM (NREM) sleep. Circadian modulation of sleep-wake behavior was provided by physiologically based interactions among these sleep-wake centers and the SCN. Model results captured stereotypical entrained human sleep-wake behavior, including typical NREM-REM cycling across the night. When effects of temporal isolation were simulated by increasing the period of the sleep-wake cycle but maintaining the ~24 h period of the circadian rhythm, model results replicated the evolution of sleep-wake patterning to internal desynchronized behavior, including transient "phase trapping." Further, varying parameters such as the strength of interactions between the SCN and sleep-wake centers allowed our model to generate a number of reported inter-individual differences in desynchronized sleep-wake behavior, including short (< 24h) sleep-wake cycles, bicircadian rhythms and irregular "phase jumping" sleep-wake behavior. Thus, this analysis predicts physiologically-plausible mechanisms to account for these inter-individual variations.

Disclosures: V. Booth: None. **R. Gleit:** None. **C. Diniz Behn:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.19/HHH11

Topic: E.08. Biological Rhythms and Sleep

Support: NSERC

Title: Serotonergic enhancement of photic phase shifts: IGL

Authors: *R. T. JEFFERS[1,2], V. M. SMITH[1,2], M. C. ANTLE[1,2,3];
[1]Psychology, [2]Hotchkiss Brain Inst., [3]Physiol. and Pharmacol., Univ. of Calgary, Calgary, AB, Canada

Abstract: In mammals, the suprachiasmatic nucleus (SCN) functions as the master circadian clock and is responsible for regulating rhythmic aspects of an organism's physiology and behaviour. The phase of these endogenously generated rhythms can be altered by environmental stimuli such as appropriately timed light exposure. Systemic administration of serotonergic drugs such as the 5-HT1A mixed agonist/antagonist BMY7378 have been shown to greatly increase the magnitude of phase shifts resulting from light exposure in the late subjective night. Exactly how BMY7378 alters the circadian clock is unknown, and recent findings suggest that it may not exert its influence solely through the SCN. The intergeniculate leaflet (IGL), like the SCN, receives direct serotonergic and retinal innervation and is involved in regulating how the circadian clock responds to light exposure. Previous research has shown that blockade of one of the major neurotransmitters released in the SCN by neurons projecting from the IGL, neuropeptide Y (NPY), can further potentiate the effects of BMY7378. However, when the IGL was lesioned, leading to the total absence of NPY expression in the SCN, administration of BMY7378 did not result in the potentiation of photic phase shifts. To further explore the mechanism through which BMY7378 exerts its influence, the current study investigated the potentiating effects of BMY7378 on light exposure in Syrian hamsters which had received selective serotonin lesions of the IGL. Following recovery, animals were given a systemic injection of either saline or BMY7378 45 minutes prior to a 40 lux, 15 minute light pulse in the late subjective night. Phase shift analysis revealed that sham-lesioned animals showed a potentiation of photic phase shifts following administration of BMY7378 when compared to saline controls. Serotonin-lesioned animals, however, did not show a significant difference in phase shifts between the BMY7378 and saline pretreatment groups. These findings indicate that serotonergic innervation of the IGL is necessary for BMY7378 to potentiate photic phase shifts.

Disclosures: R.T. Jeffers: None. **V.M. Smith:** None. **M.C. Antle:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

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Program#/Poster#: 281.20/HHH12

Topic: E.08. Biological Rhythms and Sleep

Support: NIH grant MH75968

Title: The timing of Corticosterone peak circulation affects clock gene rhythmic expression in the rat prefrontal cortex

Authors: *L. R. WOODRUFF, L. E. CHUN, L. R. HINDS, R. L. SPENCER;
Psychology/Neuroscience, Univ. of Colorado At Boulder, Boulder, CO

Abstract: It is increasingly apparent that glucocorticoids (CORT) play a pivotal role in mediating the suprachiasmatic nucleus' (SCN) coordination of circadian rhythms in peripheral tissues. Extra-SCN tissues receive sparse direct neuronal connectivity from the SCN; however glucocorticoid receptors (GR) are ubiquitously expressed in all areas of the body except for the SCN itself. This is an important distinction and suggests that CORT may be a key element in whole-body coordination of circadian rhythms. Although disrupted CORT circulation patterns, such as those associated with chronic stress and stress-related disorders, have been shown to affect clock gene rhythmicity in certain peripheral tissues, virtually no characterization of this prospect has been examined in the brain. Studying the regulation of prefrontal cortex (PFC) circadian function in particular may be useful for understanding mechanisms of extra-SCN circadian entrainment in the brain. The PFC is a known modulator of emotional and endocrine control over the stress response. The PFC also expresses a high level of GR, but it receives no direct innervation from the SCN. It is possible that alteration of CORT secretion patterns could shift or disrupt core clock gene functioning in the PFC and result in dysregulation of its function. To examine this possibility we have conducted experiments in which the presence and daily pattern of CORT exposure was manipulated in rats by adrenalectomy (ADX) ± daily CORT injection. In an initial experiment we examined *per1* and *bmal1* mRNA expression (in situ hybridization) in sham-ADX, ADX and ADX + daily CORT at ZT1 (CORT exposure pattern antiphasic to sham-ADX rats) treated male Sprague-Dawley rats. The absence of CORT did not abolish rhythmic expression of clock gene expression in the rat PFC. Surprisingly, antiphasic CORT treatment of ADX rats largely abolished rhythmic PFC clock gene expression, perhaps reflecting a disruption of the cooperative entraining influence of endogenous CORT and the SCN. Our manipulations had no effect on *per1* and *bmal1* expression in the SCN consistent with its lack of GR. In a follow-up experiment we have directly compared the effect of daily CORT treatment of ADX rats when CORT is injected at either ZT1 or ZT11 (maintained on 12:12 h LD). ADX rats were given an i.p. vehicle (n=16) or CORT (2.5 mg/kg, n=24) daily injection at either ZT1 or ZT11 for 13 days. ZT11 is approximately the time at which endogenous CORT typically peaks. One day after the last injection rats were sacrificed at ZT0, ZT6, ZT12, and ZT18.

Disclosures: L.R. Woodruff: None. **L.E. Chun:** None. **L.R. Hinds:** None. **R.L. Spencer:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.21/HHH13

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant NS05228

Title: Effect of arousal or a novel wheel on the circadian rhythm of luteinizing hormone (LH) surges and locomotor activity in estradiol-treated ovariectomized (ovx+EB) hamsters

Authors: *S. J. LEGAN[1], X. PENG[1], C. YUN[1], K. M. FRANKLIN[2], M. J. DUNCAN[2];
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Abstract: In proestrous hamsters, exposure to a novel wheel not only phase advances the activity rhythm, but completely blocks the preovulatory LH surge, which occurs 2 hours earlier the next day. Because immobilization of the wheel does not prevent these effects, they are not caused by intense locomotor activity. We hypothesized that arousal, which is associated with many nonphotic phase advancing stimuli, will block and phase advance the LH surge. Because LH surges occur daily in ovariectomized (ovx), estradiol benzoate-treated (+EB) hamsters and the effect of non-photic stimuli on such daily LH surges is not known, we examined the effect of a novel wheel and arousal in ovx+EB hamsters. Regularly cycling hamsters were housed in 14L:10D and activity rhythms were continuously monitored via infra-red detectors. After at least 7 days, the hamsters were bilaterally ovariectomized. After 2 weeks, the jugular vein was cannulated and EB or oil vehicle was injected sc. A group of intact hamsters was also cannulated on the day before proestrus. The next day (Day 1), shortly before zeitgeber time (ZT) 5 (ZT 12=lights off) the intact and ovx+oil groups were moved to the laboratory; the ovx+EB groups were moved before ZT 4 since their LH surge occurs 1 h earlier. A blood sample was obtained at ZT 5 or 4, respectively, and the hamsters were next exposed to constant darkness (DD) and either remained in their home cage or were transferred to a new cage with a running wheel, or subjected to a 2-h arousal paradigm in a new cage, i.e., exposed to 4 unique arousing stimuli for 30 minutes each. Hourly blood samples were obtained in dim red light for the next 6 h and activity was recorded hourly via an automated counter on Days 1 and 2. After the last sample, activity in DD was monitored for 1-2 weeks to analyze phase shifts. Plasma LH levels were assessed by RIA. A novel wheel had no effect on the high LH levels characteristic of ovx+oil animals. Novel wheel exposure blocked the LH surge on Day 1 in intact hamsters, and greatly attenuated this LH surge in ovx+EB hamsters. Arousal also attenuated the LH surge and delayed it 1 h ($P<0.05$). On Day 2, all LH surges were phase advanced about 2 hours. Phase advances in activity rhythms were enhanced by estradiol and arousal. In conclusion in ovx+EB hamsters, acute arousal during the normal rest phase strongly disrupts the LH surge, decreasing its magnitude and altering its timing and that of the activity rhythm, similar to novel wheel exposure. The novel wheel induced effect on LH surges is milder in ovx+EB than in intact hamsters, suggesting that either ovariectomy itself and/or the positive feedback signal elicited by exogenous estradiol treatment strengthens the neural signal for the LH surge.

Disclosures: S.J. Legan: None. **X. Peng:** None. **C. Yun:** None. **K.M. Franklin:** None. **M.J. Duncan:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.22/HHH14

Topic: E.08. Biological Rhythms and Sleep

Support: UIUC Campus Research Board Grant

NSP Graduate Fellowship

Title: The activational mechanisms underlying the impact of estrogen on the formation and expression of circadian rhythms in mice

Authors: *S. E. ROYSTON[1,2], A. G. KONDILIS[3], S. V. LORD[3], N. YASUI[4], J. A. KATZENELLENBOGEN[4], M. M. MAHONEY[3,1];
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Abstract: Estrogenic signaling shapes and modifies circadian rhythms, the disruption of which have been implicated in psychiatric, neurologic, cardiovascular, and metabolic disease, among others. While the impact of estrogen on total activity, free running period, photic phase response, activity phase angle, and the ratio of activity in the light vs. the dark (LD ratio) has been documented, the exclusively activational molecular mechanisms underlying these phenomena remain poorly characterized. To determine the activational impact of estrogen on daily behavior patterns and differentiate between the roles of ESR1 and ESR2, the primary CNS estrogen receptors, within this context, ovariectomized adult female mice were chronically administered estradiol (E), the ESR1 agonist propylpyrazole triol (PPT), the ESR2 agonist diarylpropionitrile (DPN), or cholesterol (CTL). Animals were singly housed with wheels in 12:12 LD or total darkness (DD), and wheel revolutions were recorded in 10 min bins. E administration significantly increased average daily activity compared to CTL. This effect was partially mimicked by chronic PPT, but not DPN administration, suggesting a role for ESR1, but not ESR2 in the regulation of total activity. Further, we found that E or PPT treatment consolidates activity to the dark phase, reducing the LD ratio compared to CTL. Interestingly, selective ESR2 stimulation increased the LD ratio even beyond that observed in CTL animals. Together, these data support dichotomous roles for ESR1 and ESR2 in activity consolidation across 24 hr. PPT, DPN, or E reduced the phase angle of activity onset, suggesting that ESR1 and/or ESR2

activation is sufficient for advancing the onset of activity following the transition from L to D. Similarly, the active period (alpha) was longer in animals housed in DD and treated with PPT, DPN, or E than age and litter-matched CTL animals. Conversely, the subjective day (tau) was shorter in animals administered PPT or E, but not DPN, suggesting that ESR1 activation alone is responsible for the known effect of estrogen-dependent shortening of tau. Finally, we assessed behavioral shifts in activity onset following 1 hr light pulses at various times throughout the subjective day. When pulsed in the early subjective night but no other time, activity onset was advanced in animals treated with E, PPT, or DPN compared to CTL, suggesting that ESR1 or ESR2 activation is sufficient to elicit a phase response. Importantly, we show that estrogen has strong activational effects on the temporal patterning and expression of circadian behavior, and that these effects are due to distinct mechanisms elicited by ESR1 and ESR2 activation.

Disclosures: S.E. Royston: None. **A.G. Kondilis:** None. **S.V. Lord:** None. **N. Yasui:** None. **J.A. Katzenellenbogen:** None. **M.M. Mahoney:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.23/HHH15

Topic: E.08. Biological Rhythms and Sleep

Title: Nighttime light exposure impairs affective responses in a wavelength-dependent manner

Authors: *T. A. BEDROSIAN, C. A. VAUGHN, A. GALAN, G. DAYE, Z. M. WEIL, R. J. NELSON;
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Abstract: Life on Earth is entrained to a 24-h solar cycle that synchronizes circadian rhythms in physiology and behavior; light is the most potent entraining cue. In mammals, light is detected by (1) rods and cones, which mediate visual function, and (2) intrinsically photosensitive retinal ganglion cells (ipRGCs), which primarily project to the suprachiasmatic nucleus (SCN) in the hypothalamus to regulate circadian rhythms. Recent evidence, however, demonstrates that ipRGCs also project to limbic brain regions, suggesting that through this pathway, light may have a role in cognition and mood. Therefore, it follows that unnatural exposure to light may have negative consequences for mood or behavior. Modern environmental lighting conditions have led to excessive exposure to light at night (LAN), and particularly to blue wavelength lights. The rate of depressive disorders has increased in parallel with nighttime light exposure. We hypothesized that nocturnal light exposure (i.e., dim LAN) induces depressive responses and

alters neuronal structure in rodents. If this effect is mediated by ipRGCs, which have reduced sensitivity to red wavelength light, then we predicted that red LAN would have limited effects on brain and behavior compared to shorter wavelengths. Additionally, red LAN would not induce c-Fos activation in the SCN. Our results demonstrate that exposure to LAN influences behavior and neuronal plasticity, and that this effect is likely mediated by ipRGCs. Modern sources of LAN that contain blue wavelengths may be particularly disruptive to the circadian system, potentially contributing to altered mood regulation.

Disclosures: T.A. Bedrosian: None. **C.A. Vaughn:** None. **A. Galan:** None. **G. Daye:** None. **Z.M. Weil:** None. **R.J. Nelson:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.24/HHH16

Topic: E.08. Biological Rhythms and Sleep

Title: Chronic exposure to dim nighttime light provokes depressive-like responses in mice

Authors: *C. A. VAUGHN, T. A. BEDROSIAN, Z. M. WEIL, R. J. NELSON; Neurosci., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Humans and other animals have adapted to the consistent 24-h solar cycle. The rotation of the planet on its axis and its revolution around the sun cause predictable daily light-dark cycles and seasonal patterns in day length. Modern life, however, has allowed humans to escape these naturally occurring light-dark cycles and to create schedules based on convenience. Since the invention and widespread adoption of electric lights starting ~130 years ago, humans have been increasingly exposed to artificial light at night (LAN). It seems reasonable to suggest that this unnatural light exposure may have serious effects on ecological and physiological processes that have adapted to the natural light-dark cycle. Not surprisingly, accumulating evidence suggests LAN has negative health consequences for humans, likely acting through circadian disruption. For example, shift work is associated with depressed mood and feelings of helplessness. In addition, the rate of depressive disorders in the general population has grown in parallel with increased exposure to nighttime light. LAN is a seemingly innocuous convenience, but its widespread adoption occurred prior to an understanding of circadian biology. We hypothesize that LAN is a modern source of circadian disruption leading to downstream changes in physiology and mood-related behaviors. In this study, we exposed mice for 4 weeks to either a standard 16:8 h light/dark cycle (150 lux/0 lux) or a 16:8 h light/dim light cycle (150 lux/5 lux).

Then we assessed diurnal rhythms in homecage locomotor activity and depressive-like responses in a variety of behavioral tasks. Exposure to LAN altered locomotor activity and provoked depressive-like behaviors. This study demonstrates that exposure to dim LAN has profound consequences for behavior. It is important to understand how LAN affects circadian organization and downstream physiology, particularly for urban-dwelling individuals and shift workers who are chronically exposed to disruptive LAN. Determining the types of circadian disruption provoked by LAN is critical to identifying preventative measures for these individuals.

Disclosures: C.A. Vaughn: None. **T.A. Bedrosian:** None. **Z.M. Weil:** None. **R.J. Nelson:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.25/HHH17

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant NS061804

Title: Drugs that prevent sleep also block light-induced locomotor suppression, circadian rhythm phase shifts and the drop in core temperature

Authors: *L. P. MORIN[1], P. VIVANCO[1,2], K. M. STUDHOLME[1];
[1]PSYCHIATRY, STONY BROOK UNIVERSITY MEDICAL CENTER, STONY BROOK, NY; [2]Chronobiology Lab., Dept. Physiol., Univ. of Murcia, Murcia, Spain

Abstract: When mice are briefly exposed to light during their nocturnal active phase, several simultaneous behavioral or physiological events occur. These include circadian rhythm phase shifts, a drop in core body temperature (Tc), and suppression of ongoing locomotor activity followed by sleep. The events are triggered by light, endure for a relatively fixed interval and do not require additional light for their expression. The present studies address the ability of three psychostimulant drugs to modify the light-induced responses. Methamphetamine (MA), modafinil (MOD), caffeine (CAF) or vehicle (VEH) were injected at CT11 into constant dark-housed male C57BL/j6 mice which were then exposed to a 5 min 100 uW/cm2 white light pulse or no light at CT13. Controls receiving the VEH/Light treatment showed approximately 60 min phase delays which were substantially attenuated by treatment with MA, MOD or CAF (12-15 min phase delays). Drug by itself had no effect on rhythm phase. Under a LD12:12 photoperiod, light at ZT13 induced a drop of about 1.3 oC coincident with locomotor suppression by VEH-treated mice. Both changes were generally abolished by pretreatment with any of the drugs.

Although all three drugs promoted activity increases after injection at ZT11, there was also evidence for drug-induced hypoactivity. Hyperactivity occurred during the 30 min post-injection interval for MOD, MA and CAF-treated mice, but hypoactivity was evident for CAF, but not MOD- or MA-treated, mice during 30 min of darkness (ZT12.5-ZT13) prior to the photic test stimulus. CAF acutely elevated Tc and MA acutely lowered it, but both drugs reduced Tc during the early night (ZT12.5-ZT13). The ability of the psychostimulant drugs to block the several effects of brief light exposure is not likely to be caused simply from a drug-induced locomotor activity increase. The results raise questions concerning the manner in which drugs, activity, sleep and Tc contribute to the regulation of behavioral and physiological responses to light.

Disclosures: L.P. Morin: None. **K.M. Studholme:** None. **P. Vivanco:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.26/HHH18

Topic: E.08. Biological Rhythms and Sleep

Support: 1R01NS055228

Title: Role of orexin neurons in novel wheel-induced blockade of the preovulatory luteinizing hormone (LH) surge in Syrian hamsters

Authors: *M. J. DUNCAN[1], K. M. FRANKLIN[1], X. PENG[2], C. YUN[2], S. J. LEGAN[2]; [1]Dept Anat/Neurobiol, [2]Dept Physiol., Univ. Kentucky Med. Ctr., LEXINGTON, KY

Abstract: Exposure of proestrous hamsters to a novel running wheel (NW) completely blocks the LH surge until the next day in ~70% of hamsters. We hypothesized that NW exposure activates orexin neurons, which participate in alertness, and that orexinergic neurotransmission blocks the LH surge. To test this hypothesis, we investigated the effects of 1) NW exposure on Fos expression in orexin neurons, and 2) an orexin receptor 1 antagonist, SB334867 (SB), on NW blockade of the LH surge. In Expt. 1, proestrous hamsters were exposed at zeitgeber time 5 (ZT 5, i.e., 7 h before lights- off) to either a rotating or locked NW, or remained in their home cage (HC); all were exposed to constant darkness (DD). At ZT 7, the animals were anesthetized and perfused intracardially with PBS followed by 4% PFA. Brains were removed, post-fixed, infiltrated with sucrose, and sectioned. Dual immunohistochemistry for orexin and Fos was conducted. The percentage of orexin-positive neurons expressing Fos differed among the groups ($P<0.001$; HC: 14 ± 4; locked NW: 30 ± 4; rotating NW: 42 ± 3), and was greater for rotating NW than for locked NW ($P<0.05$) and for each NW group than for HC ($P<0.02$). In Expt. 2,

hamsters were fitted with jugular cannulae and on the next day, proestrus (Day 1), blood samples were obtained at ZT 5 and i.p. injections of SB (30 mg/kg) or vehicle (100% DMSO, VEH) were given. Hamsters were placed in new cages with a rotating NW or left in HC, and exposed to DD. Blood samples were taken hourly (ZT 5-11, under dim red light) on Days 1 & 2. Plasma LH levels were determined by RIA. SB decreased the proportion of animals exhibiting NW-induced blockade of the LH surge (NW+VEH vs NW+SB, 5/13 vs 0/10 blocked, $P<0.05$). However 100% DMSO appeared to interfere with the NW effect because the NW+VEH group did not differ from the HC+VEH group (5/13 vs 1/5; $P=0.44$), whereas NW exposure usually blocks the LH surge in the majority of animals (Legan et al, J. Biol. Rhythms, 2010). Therefore, Expt. 3 was conducted using the same protocol as Expt. 2 but with 25% DMSO in hydroxypropyl-beta-cyclodextrin as the VEH. Blockade of the LH surge occurred in 8 of 10 NW+VEH hamsters, as compared with 1 of 6 HC+VEH hamsters. The effect of SB was associated with the appearance of the solutions. Thus, SB tended to decrease NW blockade of the LH surge when clear solutions were injected (4 of 6 animals, $P=0.09$), but not when the solutions were turbid (3 of 4 animals, $P=0.67$). These results indicate that NW exposure activates orexin neurons whether or not the wheel is locked, suggesting that novelty alone is effective, while novelty with running is more effective. The findings also suggest that orexin neurotransmission may participate in NW blockade of the LH surge.

Disclosures: M.J. Duncan: None. **K.M. Franklin:** None. **X. Peng:** None. **C. Yun:** None. **S.J. Legan:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.27/HHH19

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant F32MH098498

NSF Grant EEC-1028725

Title: Role of the dorsal medial habenula in the regulation of circadian rhythms

Authors: J. GILE[1], Y.-W. T. HSU[2], G. MORTON[2], S. D. WANG[2], E. E. TURNER[2], *H. O. DE LA IGLESIA[1];
[1]Biol., Univ. of Washington, Seattle, WA; [2]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA

Abstract: The epithalamic habenula has been implicated in the regulation of behaviors including motor activity and sleep. Neurons in both the medial and lateral habenula exhibit spontaneous firing that is higher during the day than night, and the lateral habenula exhibits endogenous oscillations in gene expression and neuronal activity. Here we use genetic lesions to specifically ablate neurons in the dorsomedial habenula (dMHb) in order to examine its role in the regulation of locomotor activity and circadian rhythms. Using the Cre-LoxP system, neurons in the dMHb were ablated by abolishing expression of Brn3a without affecting other regions in the habenula. dMHb-lesioned mice have reduced wheel running activity (WRA) in both a 12h:12h light-dark (LD) cycle and constant darkness (DD), but show similar levels of cage-locomotor activity compared to control animals, indicating lesions of the dMHb do not induce hyperactivity. dMHb-lesioned animals have a lengthened circadian period and re-entrain faster to a 6-hour delay jetlag. In contrast, in response to light pulses presented at CT16, they show phase shifts and increases in the expression of Per1 and cFos in the suprachiasmatic nucleus (SCN) similar to control animals. Collectively, these data suggest that the dMHb may be involved in a feedback loop that controls WRA and regulates circadian rhythms through a continuous, rather than a discrete, model of entrainment.

Disclosures: J. Gile: None. **H.O. de la Iglesia:** None. **Y.T. Hsu:** None. **G. Morton:** None. **S.D. Wang:** None. **E.E. Turner:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.28/HHH20

Topic: E.08. Biological Rhythms and Sleep

Support: ONR N000141310285

Title: Pavlovian fear conditioning following manipulation of circadian waveform

Authors: *C. L. BLOCK, E. HARRISON, M. GORMAN;
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Abstract: Shiftwork, while common, is linked to problems in performance, safety and health which reportedly cost America over $200 billion a year and are believed to be related to the misalignment of physiological rhythms with respect to the environment. One suggestion for combating challenges faced by shift workers is a temporal reorganization of activity rhythms by entrainment of the endogenous circadian pacemaker to a 24 h light:dark:light:dark (LDLD) cycle (Harrison & Gorman, 2012). The purpose of this study is to examine in a rodent model the

effects of the resulting rhythm bifurcation on learning and memory using Pavlovian fear conditioning, a paradigm that requires sleep in a photophase immediately following training (Cai). 96 C57/Bl6 mice were first entrained to either a 6:6:6:6 LDLD light cycle or a 12:12 LD control condition. While all training took place just before light transitions, half of LD animals were trained before lights off (ScotophaseFirst), and half before lights on (PhotophaseFirst). Animals were further divided into groups of 12 to be tested for contextual and cued fear 12 or 24 hours later. Importantly, for LDLD animals, each 12 h period between training and test contained both a photophase and a scotophase. Preliminary results suggest memory is intact in bifurcated mice; subsequent findings will allow us to determine how the timing of each scotophase and photophase relates to memory consolidation.

Disclosures: C.L. Block: None. **E. Harrison:** None. **M. Gorman:** None.

Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.29/HHH21

Topic: E.08. Biological Rhythms and Sleep

Support: Emergence conseil régional de Basse-Normandie

Title: Vestibular system, a new synchronization pathway of biological rhythms?

Authors: *T. MARTIN, B. MAUVIEUX, J. BULLA, B. PHILOXENE, M.-L. MACHADO, G. QUARCK, D. DAVENNE, P. DENISE, S. BESNARD;
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Abstract: All living organisms have endogenous biological timing mechanisms that regulate behavior and physiology. These biological rhythms such as body temperature (Tb) are controlled both by endogenous master clock and synchronized by external factors such as light/dark cycle (LD cycle), physical and social activities. Others parameters such as gravity has also been recently involved. Studies in dogs, monkeys and rodents exposed to hypergravity (centrifugation) show that this stimulation induces rapid fall in Tb that is mediated by the vestibular system. Fuller et al. (2002) also demonstrated that vestibular stimulation is involved by showing that centrifugation at 2G inhibits circadian rhythm of Tb in normal mice while it has no effect in mice without functional otolith system.
Instead of stimulating the vestibular system, we have studied how the loss of vestibular information via a chemical lesion influences circadian rhythm of temperature and activity in Long-Evans rats (n=18). All rats exhibited strong circadian rhythms of activity and body

temperature (τ = 24 hours) before lesion. Just after lesion, mean Tb dropped drastically by 2.5° and recovered in 4 days following an asymptotic curve but stayed lower than in sham operated rats (0.12 °). Tb circadian rhythm disappeared during 5-7 days following the lesion. The circadian period was usually recovered in all rats during the second week after the lesion. As in Tb, activity dropped during few days and its circadian rhythm was not fully recovered at day 7, probably related to a direct effect on circadian rhythm and motor symptoms of the vestibular syndrome. Sham operated rats don't present alteration of circadian rhythms of Tb and activity. Vestibular loss disrupts circadian rhythm of temperature and activity as observed after vestibular stimulation. These results confirm that the vestibular system play a role on the synchronization of circadian rhythms. Vestibular inputs might be driven by the vestibulo-latero geniculo-supra-chiasmatic neuronal pathways as hypothesized by Horowitz et al. (2005).

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Poster

281. **Entrainment of Circadian Rhythms and Sleep**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 281.30/HHH22

Topic: E.08. Biological Rhythms and Sleep

Support: CONACYT Grant no.281852

Title: The olfactory bulb and the piriform cortex anticipate feeding time during food anticipatory activity in the rat

Authors: *M. PABELLO[1], M. L. MORENO[1], E. MEZA[2], M. CABA[2];
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Abstract: It is well established that the olfactory bulb (OB) has a circadian clock, independent of the suprachiasmatic nucleus, the master clock in mammals. Moreover, OB drives rhythmic oscillations in the piriform cortex (PC), which reinforce the role of the OB as a pacemaker. However, very little is known about the functional significance of OB and PC oscillations. In present experiments we explored whether OB and PC can be entrained under conditions of food restriction. To this aim we used male adult rats under a restricted feeding schedule. Subjects were maintained under a 12/12 h light/dark cycle (lights on at 07:00 h = ZT0) and divided into three groups. Group 1: Ad libitum (AL), with free access to food. Group 2: Restricted feeding (RF), with restricted food access from ZT5-ZT7 for three weeks. Group 3: Restricted feeding-

fast (RF-F), similar to Group 2, then food was removed for two days, to explore the possible persistence of oscillations. Subjects were euthanized at ZT1, ZT5, ZT7 and ZT13. OB and brains were removed and sectioned coronally in a cryostat (50 μm) and processed by immunohistochemistry for FOS protein, product of the c-fos gene, as an indicator of neural activation. In the OB we explored Glomerular, Granular and Mitral cell layers. The piriform cortex was explored at the level of the lateral olfactory tract. AL subjects expressed low levels of FOS in three OB layers and PC, at all times explored except at ZT13, i.e, around the onset of night, where FOS values were significantly higher ($P<0.01$) in comparison to previous time points. However, RF subjects showed a completely different pattern. FOS was low at ZT1 but at ZT5, at time of feeding, showed a sharp increase, which further increase at ZT7. Then values sharply decrease at ZT13. values at ZT5 and ZT7 were significantly higher ($P<0.01$) in RF, in comparison to corresponding values in AL subjects in the three OB layers and in the PC. FOS in RF-F subjects follows a similar pattern as that of RF subjects, except in PC where values do not drop at ZT13. We conclude that both OB and PC are entrained by scheduled feeding and show a similar pattern of activation. Moreover, both structures "anticipate" feeding time during food anticipatory activity when subjects show a state of high arousal in advance of mealtime.

Disclosures: M. Pabello: None. **M.L. Moreno:** None. **E. Meza:** None. **M. Caba:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.01/HHH23

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: FDA/NCTR Protocol E7460

Title: A novel approach for the determination of cytotoxicity in an *In vitro* blood-brain barrier model

Authors: *Q. GU[1], S. LANTZ[1], H. ROSAS-HERNANDEZ[1], E. CUEVAS[1], S. SARKAR[1], L. SCHMUED[1], V. KRAUTHAMER[2], M. G. PAULE[1], S. F. ALI[1];
[1]Natl. Ctr. For Toxicological Research/FDA, Jefferson, AR; [2]Ctr. for Devices and Radiological Health/FDA, Silver Spring, MD

Abstract: The blood-brain barrier (BBB) plays a crucial role in the maintenance of the chemical environment of the central nervous system by controlling the entrance and exit of substances from blood to brain and *vice versa*. A disruption of the integrity of the BBB can cause brain infection, inflammation, epilepsy, and neurodegenerative disorders. Primary cultures of

confluent brain micro-vessel endothelial cells (BMVEC) have been established as a useful model for the study of BBB physiology, biochemistry, and molecular events induced by internal and external factors. Here we report a novel approach for the labeling of toxicant-induced cell death in cultured rat BMVEC using Fluoro-Jade C (FJ-C). Fluoro-Jade stains have been extensively utilized in *in vivo* studies of neurotoxicity where they have been shown to label dead and dying neurons, but have never been applied in *in vitro* BBB model systems. Here, four different toxicants (cadmium, thimerosal, sodium nitroprusside, and silver nanoparticles) served as positive controls with the culture medium serving as a negative control in these proof-of-concept experiments. All toxicants elicited a significant, dose-dependent increase in the number of Fluoro-Jade C labeled cells. The toxicant-induced cell death, as determined by the Fluoro-Jade C labeling, was further confirmed by two conventional cytotoxicity tests, the lactate dehydrogenase (LDH) and XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assays. Compared to these two conventional assays, the F-J C-labeling appears to have the following advantages: it is simpler, faster, has higher sensitivity, higher specificity, and a larger dynamic range. Together, these results suggest that the Fluoro-Jade C-based labeling approach can provide a simple and rapid assessment of cell death in the BMVEC-BBB model and has the potential to lead to high-throughput screening and analyses of cytotoxicity *in vitro*.

Disclosures: Q. Gu: None. **S. Lantz:** None. **H. Rosas-Hernandez:** None. **E. Cuevas:** None. **S. Sarkar:** None. **L. Schmued:** None. **V. Krauthamer:** None. **M.G. Paule:** None. **S.F. Ali:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.02/HHH24

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: The effect of chronic treatment with atypical antipsychotic drugs to P-glycoprotein in the mice brain

Authors: *T. WATANABE, K. OSADA, T. HAGA, Y. OGAWA, A. MUTO, A. TAGUCHI, T. YANAGIDA, M. NAKANO, Y. SASUGA, N. YAMAGUCHI;
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Abstract: P-glycoprotein is a 130-kDa adenosine triphosphate (ATP)-dependent drug transport protein that is abundantly distributed in the apical side of brain capillary endothelial cells forming the tight junctions of the blood-brain barrier (BBB). P-glycoprotein has been demonstrated to influence the absorption, distribution, and elimination of many commonly used

drugs. It has, furthermore, been shown that P-glycoprotein influences the distribution of drugs across the BBB. The location of P-glycoprotein at the BBB is of importance for the delivery of psychotropic drugs such as antidepressant and antipsychotic medications. Among these drugs are the antidepressants nortriptyline, citalopram, sertraline, and fluvoxamine, the antipsychotic drugs olanzapine and risperidone, as well as the substrates for P-glycoprotein. But we do not know how the P-glycoprotein function after the chronic treatment with atypical antipsychotic drugs in the brain. Then we investigated which chronic treatment with the atypical antipsychotic drugs as the substrates for P-glycoprotein was changed the function of P-glycoprotein in the brain. We examined that the expression of RNA P-glycoprotein after chronic treatment with the antipsychotic drugs and compared with before treatment. C57BL/6N mice (weighing 20-25 g) were orally administrated of 10mg/kg/day aripiprazole once daily for six weeks. To quantify the amount of mRNA in mice brain, we performed real-time PCR (7500 Fast Real-Time PCR System)by using TaqMan Fast Universal PCR Master Mix (life technologies). A PCR reaction mixture of 20µl containing 10µl of TaqMan Fast Universal PCR Master Mix, 9 µl of cDNA and 1 µl TaqMan Gene Expression Assays. Aripiprazole (P-glycoprotein inhibitor) induced the increase of P-glycoprotein expression. However aripiprazole inhibited the export of digoxin (P-glycoprotein substrate) by P-glycoprotein from brain to blood, then the brain concentration of digoxin was inceased.

Disclosures: T. Watanabe: None. **K. Osada:** None. **T. Haga:** None. **Y. Ogawa:** None. **A. Muto:** None. **A. Taguchi:** None. **T. Yanagida:** None. **M. Nakano:** None. **Y. Sasuga:** None. **N. Yamaguchi:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.03/HHH25

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01-DA029121-01A1

ARDF

Title: Mechanistic insights into altered glycaemia-induced blood-brain barrier dysfunction *In vitro*

Authors: *R. K. SAJJA, S. PRASAD, P. NAIK, L. CUCULLO;
Pharmaceut. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX

Abstract: Of the many microvascular complications resulting from diabetes-dependent altered glycaemia, the blood-brain barrier (BBB) dysfunction is the most critical, as it exacerbates a host of brain disorders. In this study, we assessed the impact of acute/chronic hyper or hypoglycemic conditions on brain microvascular endothelial cell (HBMEC/D3 cell line) physiology, which may compromise the function and integrity of the BBB. Parallel monocultures of D3 cells (between passages 29-35) were exposed to either hyper or hypoglycemic conditions (35 or 2.2 mM D-glucose respectively, in D3 cell/pericyte cocktail medium [1:1]). Following 24 h (acute) or 72 h exposure, the expression of various glucose transporters and tight junction proteins were analyzed by immunocytochemistry and western blot analyses and were compared to controls (normoglycemic conditions ≈ 5.5 mM D-glucose). Media samples from all cultures collected at 24 h and 72 h were further assayed for VEGF and PDGF-BB levels. Side by side comparison of immunofluorescence data at 24 h and 72 h showed a consistent up-regulation of GLUT-1 and SGLT1 under hypoglycemic condition, while hyperglycemia induced a remarkable decrease of these transporters (vs. control). Relative to control condition, hyperglycemia induced a marginal increase of insulin-β receptor expression which was instead decreased in hypoglycemic cultures. Exposure to either hyper or hypoglycemia resulted in a significant down-regulation of claudin-5 and remarkable changes in distribution patterns of P-gp. In addition, both hyper and hypoglycemia elicited a significant increase in Nrf-2 (a primary regulator of oxidative stress) expression. Moreover, a progressive elevation in VEGF levels was observed at 24 and 72 h following hyper or hypoglycemia (vs. control). Interestingly, exposure to hypoglycemic conditions (but not hyperglycemia) significantly reduced PDGF-BB levels. These data suggest that hyper and hypoglycemic conditions induce a progressive dysfunction of brain microvascular endothelial cells which could be prodromal to BBB impairment and involve oxidative stress damage. Additional studies are currently in progress (BBB integrity and corresponding functional assessments in vitro using humanized flow-based and static BBB co-culture models) to validate our results and dissect out the differential impact of acute vs. chronic altered glycemic conditions on BBB function. (Supported by NIH/NIDA R01-DA029121-01A1 and A.R.D.F. to Dr. Luca Cucullo)

Disclosures: R.K. Sajja: None. **S. Prasad:** None. **P. Naik:** None. **L. Cucullo:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.04/HHH26

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH T32 GM007546 Clinical Pharmacology Training Grant

Title: Regulation of steroid disposition and animal aggression by Mdr1 and BCRP

Authors: *R. MUNJI[1], R. DANEMAN[1], R. BAINTON[2];
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Abstract: ABC efflux transporters expressed at the BBB protect the CNS from xenobiotic toxicity. However, the ability of ABC transporters, P-glycoprotein and BCRP, to efflux a myriad of endogenous compounds suggests that these transporters may function to limit the biological activity or toxicity of their endogenous substrates in the CNS. Both P-glycoprotein and BCRP have been shown to transport biologically potent substrates including steroids, neuropeptides and vitamins. Thus, we hypothesize that defects in P-glycoprotein or BCRP activity could lead to aberrant CNS signaling and potentially detrimental effects on neural function. In accord with this hypothesis, we observe abnormal aggression in male Mdr1-BCRP knockout mice. Male Mdr1-BCRP knockout mice exhibit increased responses when compared to wildtype mice in the resident-intruder inter-male aggression test. To characterize the roles of P-glycoprotein and BCRP in regulating CNS function, we are analyzing the disposition and activity of P-glycoprotein and BCRP substrates known to modulate animal behaviors. Our preliminary data suggests that sex steroid concentrations are altered in Mdr1-BCRP knockout mice. These findings implicate efflux transport as an important mechanism for regulating endobiotic disposition and CNS homeostasis. Moreover, our results reveal a potentially undesirable side effect of modulating P-glycoprotein or BCRP activity for CNS drug delivery.

Disclosures: R. Munji: None. **R. Daneman:** None. **R. Bainton:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

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Program#/Poster#: 282.05/HHH27

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: VA Grant 1I01BX001657-01 to CHL

Title: Arachnoid cell morphology/physiology in fibroblast co-cultures

Authors: *C. H. LAM[1], E. HANSEN[2];
[1]Neurosurg., Univ. of Minnesota, MINNEAPOLIS, MN; [2]VA Med. Ctr., Minneapolis, MN

Abstract: Introduction: Fibroblast is pervasive in the setting of injury to many organs. Its invasion into the arachnoid tissue causes scarring, adhesion of the brain, and obstruction of cerebrospinal fluid outflow. We studied the effects of fibroblast grown in close proximity to the arachnoid.
Methods: An immortalized rat cell line was used in conjunction with rat fibroblast primary cultures. Transport properties, morphology, and growth characteristics were analyzed. Semi permeable transwell membranes with monolayers of cocultured arachnoid and fibroblast cells (25%, 50%, 75%, and 100% ratio of arachnoid to fibroblast) were compared using TEER, permeability of mannitol, morphology, and mortality.
Results: The barrier function (TEER) of cell monolayers decreased with the invasion of fibroblast. Similarly the permeability of mannitol significantly increased as the ratio of arachnoid to fibroblast decreased. The morphology of arachnoid cells did not change in fibroblast co-cultures, however arachnoid cells died at a significantly higher rate when in close proximity to fibroblasts compared to arachnoid cell monocultures alone.
Conclusion: We demonstrate that fibroblast alter arachnoid cell behavior including barrier capability and viability. Fibroblasts may influence arachnoid cell's mannitol transport via soluble factors. While the arachnoid cells did not change morphologically, over time, the cells became overwhelmed, mimicking the pathologic event of scarring.

Disclosures: C.H. Lam: None. **E. Hansen:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Canadian Foundation for Innovation

CIHR - Catalyst Grant

Canada Research Chair Program

NSERC Discovery Grant

Title: Regional disparities in cerebral blood flow velocity changes induced by visual vs. executive function tasks

Authors: *T. BURNETT, P. AINSLIE, P. VAN DONKELAAR;
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Abstract: Neurovascular coupling (NVC) is the process by which neural activity drives changes in cerebral blood flow velocity (CBFv). Visual stimulation is traditionally used to increase neural activation of the visual cortex, thus, causing an NVC response in posterior cerebral artery velocity (PCAv). How cognitive tasks increase the neural activity of the frontal cortex thereby eliciting an NVC response in the middle cerebral artery velocity (MCAv) are poorly understood. The aim of this study was to use a traditional visual stimulation paradigm to elicit an NVC response in PCAv, and compare that with NVC responses in MCAv during an executive function task. Participants were fitted with two 2MHz transcranial Doppler ultrasound probes to measure CBFv simultaneously in the right PCA and left MCA. In addition, end tidal CO_2 (PCO_2), and mean arterial pressure (MAP) were also monitored. Measurements were continuously made under three conditions: i) Passive vision in which the eyes were alternately opened and closed 5 times for 40 and 20 seconds, respectively; ii) Active vision in which the participants read a passage during the eyes open segments; and iii) Executive function in which the participants completed a task-switching task during which they alternated responding congruently and incongruently to a spatial target. Results showed that passive and active vision resulted in a larger NVC response in PCAv than the task-switching task which showed a significantly larger NVC response in MCAv. The changes induced by the task-switching task occurred despite the fact that there were robust reductions in PCO_2. Taken together, these results demonstrate regional differences in CBFv in response to neural activation across two broad categories of tasks that differentially engage frontal and posterior brain regions.

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Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.07/HHH29

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Centre for Stroke Recovery

Heart Stroke Foundation of BC and Yukon

Canadian Institute for health research

Title: Optogenetic activation of mouse cortical inhibitory interneurons is sufficient to increase local blood flow

Authors: *E. ANENBERG[1], J. LEDUE[2], T. H. MURPHY[2];
[1]Neurosci., [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Interplay between various cell types in the brain orchestrates the recruitment of blood flow in accordance with local activity. Coupling alterations in blood flow, and presumably oxygen delivery, with levels of neuronal activity is essential for normal brain function; however, the underlying mechanisms involved in this regulation are unclear. Studies suggest a role for astrocytes, pericyctes, pyramidal neurons and interneurons in controlling cerebral blood flow. Here we use optogenetic techniques, cell type selective excitation, to investigate whether direct activation of inhibitory interneurons can alter blood flow. By imaging through a polished reinforced thinned skull window, we have observed that direct photostimulation of inhibitory cells in vivo in VGAT-mhChR2-YFP BAC transgenic mice alters levels of cortical blood flow and activity. With 3.3 mW of 473nm light, 100ms of 100Hz 5ms stimulation led to a 9.15±1.08% increase in the laser speckle signal within 1.9 s (n=3). Similar increases in blood flow assessed by laser speckle (10.04±0.03%) were achieved with 4 s of sensory stimulation (n=2). Intrinsic optical signals were used to confirm that stimulation led to brain activation. The initial dip of the intrinsic signal, an indirect measure of activity composed largely of the deoxygenation of hemoglobin (Hb) (Frostig et al. 1990) , reached its minimum of -0.021±0.018 ΔR/R% within 1.2 s (n=5). Signal overshoot, which is proposed to consist largely of HbO2 increase and deoxyHb decrease (Malonek and Grinvald, 1996) reached a maximum of 0.205±0.032 ΔR/R% within 3.2 s (n=5). Sensory stimulation led to an initial dip of similar amplitude to direct optogenetic stimulation (-0.020±0.004 ΔR/R% within 1.2 s) but a smaller overshoot (0.024±0.008 ΔR/R% within 4.8 s) (n=3). These data suggest that the activation of interneurons is sufficient to mediate elevations in cortical blood flow.

Disclosures: E. Anenberg: None. **J. LeDue:** None. **T.H. Murphy:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Research Center Program of IBS (Institute for Basic Science)

Korean Health Technology R&D Project (A110097)

Title: Effective cerebral blood volume enhancement by flexible, transparent, and non-cytotoxic graphene electric field stimulator

Authors: *C. HEO[1], S. LEE[1], A. JO[2], S. JUNG[2], M. SUH[2], Y. LEE[1];
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Abstract: The global population is aging; thus, the risk for cerebrovascular diseases and associated deaths is rapidly increasing. The enhancement of cerebral blood volume (CBV) in a targeted area without causing side effects is a primary strategy for treating cerebral hypoperfusion. Here, we report a new non-pharmaceutical, and non-vascular surgical method to increase CBV. A skin-like biocompatible graphene electrical field stimulator (GEFS) is fabricated with an ultra-thin electrode of graphene, two-dimensional honeycomb arrangement of carbon atoms which yields superb electrical conductivity, high transmittance, and excellent flexibility. GEFS was placed directly onto the cortical brain and anon-contact electric field was applied at a specific local blood vessel. Effective CBV increases in the blood vessels of mouse brains were directly observed from in vivo optical recordings of intrinsic signal (ORIS) imaging. The CBV was significantly increased in arteries of the stimulated area, but neither tissue damage nor unnecessary neuronal activation was observed. No transient hypoxia was observed. This technique provides a new method to treat cerebral blood circulation deficiencies at local vessels and can be applied to brain regeneration and rehabilitation.

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Poster

282. **Blood Flow and Blood Brain Barrier**

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: European Research Council

Fondation Leducq

MRC PhD studentship

Wellcome Trust

Title: Capillaries dilate in brain slices and *In vivo* in response to neuronal activation, and may initiate the vascular response

Authors: *C. N. HALL[1], C. REYNELL[1], B. GESSLEIN[2], N. HAMILTON-WHITAKER[1], A. MISHRA[1], D. ATTWELL[1];
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Abstract: Neuronal activity is energetically expensive, so when different parts of the brain are active, blood flow increases to these active regions. This is thought to occur when glutamatergic signalling produces second messengers in other neurons or astrocytes, leading to dilation of arteriolar smooth muscle cells. This increases arteriole diameter and therefore blood flow to the region fed by a given vessel. Recent work, however, suggests that capillaries may also be important for controlling neuronal energy supply, as application of neurotransmitters to brain slices causes capillaries to constrict and dilate near pericyte cell bodies (Peppiatt et al., 2006, Nature 443, 700-4,). Here, we demonstrate that capillaries also dilate in brain slices when neurons are activated. This dilation, either in response to applied glutamate or neuronal stimulation, occurs via prostaglandin E2 acting on EP4 receptors. Capillaries also dilate in vivo in somatosensory cortex during whisker stimulation. This dilation often occurs before the feeding arteriole response, demonstrating that the capillary dilation is an active response due to relaxation of capillary pericytes, rather than a passive effect of altered blood pressure due to upstream arteriole dilation. Importantly, this result implies that capillary pericytes may be the vascular cells that first sense local increases in neuronal activity and initiate the signal to increase blood flow to an active region. The spatial resolution of changes in blood flow in response to local changes in neuronal activity may, therefore, be much more precise than previously thought.

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Poster

282. **Blood Flow and Blood Brain Barrier**

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: University of Wisconsin-Madison School of Pharmacy

University of Wisconsin-Madison Graduate School

Michael J Fox Foundation for Parkinson's Research

Wisconsin Alzheimer's Disease Research Center (P50-AG033514)

UW Institute for Clinical and Translational Research

Title: Intranasal targeting of antibodies to the central nervous system: A potential noninvasive strategy for chronic immunotherapy against brain disorders

Authors: *J. J. LOCHHEAD[1], N. KUMAR[1], C. VIESSELMANN[1], R. THORNE[1,2,3,4]; [1]Pharmaceut. Sci., Univ. of Wisconsin, Madison, WI; [2]Clin. Neuroengineering Training Program, [3]The Ctr. for Neurosci. and Neurosci. Training Program, [4]Cell. and Mol. Pathology Grad. Program, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Antibody therapeutics have shown efficacy in pre-clinical models of Alzheimer's disease, Parkinson's disease, and glioma. Intravenously administered anti-beta-amyloid immunoglobulin G (IgG) is currently in clinical trials as a disease-modifying therapy for Alzheimer's disease. This approach may primarily work through the sequestration of beta-amyloid by circulating antibodies (i.e. a "peripheral sink"); however, there is also evidence that antibodies can prevent beta-amyloid oligomerization, facilitate amyloid disaggregation and stimulate Fc-mediated phagocytosis by microglia within the CNS provided sufficient amounts of IgG are able to access the brain. Achieving reliable, chronic CNS delivery for macromolecules like IgG (150 kDa) has been a major unmet challenge because the blood-brain barrier severely restricts brain entry from the systemic circulation. Previously, several proteins have been shown to directly access the CNS through pathways associated with the olfactory and trigeminal nerves following intranasal (IN) administration (Lochhead & Thorne. *Adv Drug Deliv Rev*, 2012). Rapid delivery (30-60 min) of proteins to widespread areas of the CNS following IN administration has suggested a convective (bulk flow) process is involved, possibly along cerebral perivascular spaces (Thorne et al. *Neuroscience*, 2004 & 2008). Although a few studies have indicated antibodies may reach the brain after IN application, quantitative data regarding the resulting IgG distribution in the CNS has not been available. Our previous work suggested that matrix metalloproteinase-9 (MMP-9), a physiologic, nasal epithelial tight junction modulator could enhance the access of fluorescently labeled dextrans, albumin (67 kDa) and IgG to the perivascular spaces of cerebral blood vessels following IN delivery (Lochhead & Thorne. *SfN Abstracts*, 2012). We hypothesized that IN MMP-9 pretreatment would increase antibody delivery to the brain by enhancing IgG transport across the nasal epithelium and allowing it greater access to pathways connecting the nasal submucosa to the brain. In the present study, we intranasally administered MMP-9 and ^{125}I-IgG to rats and then measured ^{125}I-IgG distribution and concentration throughout the CNS using autoradiography and gamma counting. Our results demonstrate rapid delivery of ^{125}I-IgG to widespread areas of the CNS following IN administration. These findings provide evidence that passive immunization using the intranasal route may allow for noninvasive, specific targeting of pathologic antigens residing within the CNS, offering several advantages over other systemic routes for chronic immunotherapy.

Disclosures: J.J. Lochhead: None. **N. Kumar:** None. **C. Viesselmann:** None. **R. Thorne:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant 5RO1NR003521

2R42NS065515-02A1

Title: Novel recombinant T cell receptor ligand RTL 1000 is effective in the murine model of female stroke

Authors: J. M. PALMATEER[1], J. PAN[1], T. SCHALLERT[2], H. OFFNER[3,4,5], *P. D. HURN[6,1]; [1]Section of Neurobiology, Col. of Natural Sci., [2]Dept. of Psychology, Univ. of Texas at Austin, Austin, TX; [3]Neuroimmunology Res., Veteran's Affairs Med. Ctr., Portland, OR; [4]Dept. of Neurol., [5]Dept. of Anesthesiol. and Perioperative Med., Oregon Hlth. & Sci. Univ., Portland, OR; [6]Office of Hlth. Affairs, The Univ. of Texas Syst., Austin, TX

Abstract:
Inflammation is an important mechanism by which the brain is damaged after ischemia but also an important element in healing and repair. Little is known about the role of T lymphocytes and adaptive immunity, but recent reports indicate that some subsets of T cells alter ischemic outcomes by a variety of mechanisms. We studied a recombinant, humanized T cell receptor ligand, RTL 1000, currently under evaluation as a clinical therapy for multiple sclerosis and which previously has been shown to reduce tissue damage after middle cerebral artery occlusion (MCAO) in male mice. We now test the hypothesis that RTL 1000 would be effective in DR2*1502 TG female mice with humanized T cell receptors, both by reducing histological damage and by improving complex behavioral recovery. Female DR2 mice (18-21g) were treated with MCAO (60 min) or sham surgery, and then recovered for 96h or 2 weeks. Ischemic cerebral perfusion was measured by laser Doppler flowmetry, and was not different between MCAO-treated groups. RTL or vehicle (VEH) was administered at 3h, 24h, 48h and 72h post-occlusion. Infarction volume (TTC staining, n=15 per group) was: Cortex: P value: 0.005; RTL1000 19.89% (+/- 6.25), Vehicle 44.24% (+/- 4.99), Striatum: P value: 0.002; RTL 1000 24.17% (SEM: +/- 6.81), Vehicle 52.4% (+/- 4.74), Infarcted hemisphere/contralateral hemisphere: P value: 0.009; RTL1000 13.94% (+/- 4.07), Vehicle 28.29% (+/- 3.03). No

differences were found for
mortality rate, intra-ischemic cerebral perfusion, body weight during recovery or post-operative general health score between groups. Reduction of infarction to near zero was observed in RTL (n=7). We conclude that RTL 1000 strongly improves ischemic outcomes in young, reproductively intact, female mice.

Disclosures: J.M. Palmateer: None. **J. Pan:** None. **T. Schallert:** None. **H. Offner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Artielle ImmunoTherapeutics, Inc.. **P.D. Hurn:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH NINDS AREA 1R15NS062404-01A2

Title: MCT1 function in cerebral microvascular endothelial cells is decreased by cAMP through a mechanism that reduces trafficking to the cell surface

Authors: *J. P. SMITH, L. LI, N. LAVOY, M. J. VELASQUEZ, A. L. UHERNIK; Colorado State Univ.-Pueblo, PUEBLO, CO

Abstract: Monocarboxylic Acid Transporter 1 (MCT1) in cerebral microvascular endothelial cells is the main mechanism for monocarboxylic acids to cross the blood-brain barrier giving it a major role in maintaining cerebral monocarboxylic acid homeostasis in health and development. It also controls lactic acid flux from brain to blood during lactic acidosis, which is foundational to the etiology of brain damage during stroke and brain injury. Therefore, understanding the detailed mechanisms that regulate cerebrovascular MCT1 could provide important new targets for interventions that could modulate lactic acidosis to improve outcomes. Our previous studies showed cAMP-dependent regulation of Mct1 function in rat brain endothelial cells involves a mechanism that reduces the transporter's level of expression and phosphorylation on the plasma membrane. Associated with this regulation, caveolin-1 was dephosphorylated and Mct1 translocated to caveolae. However, whether caveolar dependent endocytosis or some other mechanism was responsible for reduced membrane expression of Mct1 was not determined. To investigate this, we evaluated changes in Mct1 function, total cellular phosphorylation, rates of

membrane insertion, and endocytic rates in rat brain endothelial cells that were briefly exposed to 8-Br-cAMP, and inhibitors of vesicular trafficking, methyl beta cyclodextrin (MBCD) and dynasore. Each of these agents reduced Mct1 function, reduced Mct1 trafficking to the plasma membrane, and increased the colocalization of mCherry-tagged Mct1 with GFP-tagged caveolin-1. 8-Br-cAMP increased endocytosis of 70 kD dextran, but reduced colocalization of Mct1 with the endocytosed material. In contrast, dynasore and MBCD both decreased endocytosis of 70kD dextran. Phosphorylation of total cellular Mct1 was increased by 8-Br-cAMP. Combined these results show cAMP regulates Mct1's surface expression in brain endothelial cells through a pathway in which phosphorylation of transporters causes reduced delivery to the plasma membrane, while dephosphorylated transporters remain at the surface. cAMP dependent translocation of Mct1 to caveolin-1, which becomes dephosphorylated, and the direct inhibition of Mct1 by MBCD, supports a mechanism of Mct1 inactivation in a pathway involving caveolin-1, but possibly not caveolar dependent endocytosis. Because inhibitors of endocytosis strongly inhibited Mct1 function, our overall results show a mechanism in which the dynamics of Mct1 surface trafficking not only regulate its function but are essential to it.

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Poster

282. **Blood Flow and Blood Brain Barrier**

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant OD006831

NIH grant EB003832

Title: Probing neurovascular coupling via natural and driven vasomotion in the awake mouse

Authors: *C. MATEO[1], P. S. TSAI[1], A. Y. SHIH[3], D. KLEINFELD[1,2];
[1]Physics Dept, [2]Neurobio., UCSD, La Jolla, CA; [3]Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Neuronal activation by sensory stimulation or brain microstimulation leads to a change in vascular tone that forms the basis of functional magnetic resonance imaging (fMRI) and intrinsic optical imaging. Yet, in the absence of external stimulation or a specific cognitive task, spontaneous cortical activity persists during what has been referred to as the "resting brain

state". Interestingly, infra-slow oscillations of the fMRI signal (< 0.2 Hz) in humans and monkeys correlate with spontaneous fluctuations in neuronal activity (Schölvinck, Maier, Ye, Duyn & Leopold, PNAS, 2010). We suggest that this low frequency signature may provide a means to study neurovascular coupling. Toward this goal, we combined functional two-photon imaging of cortical hemodynamics with electrophysiological recordings and optogenetics in awake, head-fixed mice. We achieve non-invasive optical access over the superficial layers of the somatosensory cortex with a thin-skull cranial window. Neuronal activity across the window was assessed using differential local field potential recordings from a pair of electrodes that spanned the window.
Consistent with a previous report (Drew, Shih & Kleinfeld, PNAS, 2011), we observe infra-low frequency dilations, at < 0.2 Hz, in surface and penetrating arterioles but not in veins. We further observe strong spectral coherence at these infra-low frequencies between the envelope of the gamma rhythm band of neuronal activity, nominally 30 to 80 Hz, and the diameter of the arterioles (n = 8). This analysis also reveals that changes in the envelope of the gamma rhythm lead the dilation of all imaged arterioles by several seconds, suggesting that the gamma rhythm serves as a predictor of slow vasodynamics in the neocortex. As one control, the observed coupling between the gamma rhythm and vasomotion was disrupted by isoflurane anesthesia, ~3 % in oxygen, which maximally dilates brain arterioles.
To probe the possibility of a causal effect of fluctuations in the local blood supply on neuronal activity, we use focused laser light to periodically dilate individual arterioles via the hyperpolarization of smooth muscle cells that genetically express halorhodopsin (n = 2). Data from these experiments will allow us to assess the potential role of changes in local perfusion on spontaneous ongoing activity.

Disclosures: C. Mateo: None. **A.Y. Shih:** None. **D. Kleinfeld:** None. **P.S. Tsai:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.14/HHH36

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: VA Merit Review Grant # 1I01BX001657-01

Title: Arachnoid cell motility in fibroblast co-cultures

Authors: *E. A. HANSEN[1], C. YUAN[2], C. LAM[2];
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Abstract: Introduction: The barrier between the CSF and the blood is in part formed by the arachnoid cells. Models of arachnoidal tissue however are limited. We have developed an immortalized arachnoid cell line derived from the rat with barrier properties indicative of a CSF-blood barrier model and observe arachnoid cell aversive movement in fibroblast cell cultures.
Method: Rat cells were immortalized by LgTAg vector as described previously and labeled with GFP. GFP labeled arachnoid cells were plated at 1:1 ratios with fibroblast and irradiated fibroblast. Arachnoid cell motility was determined by taking a photograph using a florescent microscope with incubator every 5 minutes for up to 72 hours. Movies were created allowing single cells to be tracked and monitored for distance moved, directional movement (i.e. away from fibroblasts), and cell mortality.
Results: Arachnoid cells moved significantly longer distances when grown in fibroblast co-cultures compared to irradiated fibroblast co-cultures and arachnoid cell monocultures. Arachnoid cells died at the highest rate when grown with non-irradiated fibroblast, and had lower mortality when grown with irradiated fibroblast and arachnoid controls. Arachnoid cells appeared to move away from fibroblast cells when in close proximity (i.e. less then 5µm).
Conclusion: We demonstrate that fibroblast alter arachnoid cell behavior including movement and mortality. Development of an in vitro model for the arachnoid granulation and one that models scarring will enhance our understanding of transport in the tissue and provide the basis for the development of an arachnoid equivalent.

Disclosures: E.A. Hansen: None. **C. Yuan:** None. **C. Lam:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.15/HHH37

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH

Title: Identification of candidate genes acting as Notch-regulators and their role under hypoxic conditions

Authors: *T. STOBDAN[1], D. ZHOU[1], G. HADDAD[1,3,2];
[1]Pediatrics, [2]Neurosci., Univ. of California San Diego, La Jolla, CA; [3]Rady Children's Hosp., San Diego, CA

Abstract: The evolutionarily conserved Notch signaling is one of the most important regulators of development. Our previous studies have found that activation of Notch signaling in the Eaat1-

positive glial cells plays a critical role in the developmental process under hypoxic conditions by showing a remarkable increase in hypoxia tolerance. To study the genetic interactions that underlie this Notch-activation that confers protection during hypoxia and development, we used UAS/Gal4 system and generated a Drosophila melanogaster line with constitutive Notch activation by specifically overexpressing Notch intracellular domain (the functional domain mediating Notch function) in the Eaat1-positive glial cells (i.e., the EN line). In the current study, we crossed this UAS-NICD/Gal4 EN line with UAS-RNAi lines targeting specific gene(s). In such cross, the targeted gene will be down-regulated on a Notch-activation background and this will serve to delineate any interaction between Notch and the targeted gene. Prioritization of candidate genes (n=66) was based on gene expression profiling of Drosophila strain that lives perpetually in an extremely low-oxygen environment. Our assay was to investigate the eclosion rates of Drosophila embryos developing under hypoxic (5% O2) and room air (21% O2) conditions. Preliminary results indicate several candidate genes, when suppressed in glial cells, showed a protective role to severe hypoxia. In contrast, down-regulating a gene encoding a ribosomal protein in glia was found to be deleterious under both room air and hypoxic conditions. Interestingly, this deleterious effect was rescued by Notch activation under both hypoxic and room air conditions. In conclusion, we have studied the interactions between Notch and other genes in Eaat1-positive glial cells and have identified interesting Notch interactive genes that play important roles in development under hypoxic conditions.

Disclosures: T. Stobdan: A. Employment/Salary (full or part-time):; Univ of California San Diego. **D. Zhou:** A. Employment/Salary (full or part-time):; University of California San Diego. **G. Haddad:** A. Employment/Salary (full or part-time):; Univ of California San Diego.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: R01 NS076628 (NINDS)

R01 NS063226 (NINDS)

UL1 RR024156 (NCATS)

NSF 0954796

Human Frontier Science Program (HFSP)

Title: Are resting state and stimulus-evoked neurovascular coupling different?

Authors: *Y. MA, A. RAYSHUBSKIY, S. H. KIM, D. TIMERMAN, M. G. KOZBERG, E. M. C. HILLMAN;
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Abstract: Neurovascular coupling refers to the tight link between changes in neuronal activity and changes in local cerebral blood flow and cerebral blood oxygenation. Functional neuroimaging techniques such as functional magnetic resonance imaging (fMRI) rely on the assumption that the relationship between the hemodynamic response and local neural activity is linear. Several studies have shown correlations between the stimulus-evoked hemodynamic response and both local field potential and local multi-unit activity.
More recently, resting state functional connectivity mapping has been adopted as a tool to assess the brain's innate neuronal networks by analyzing regional correlations in spontaneous fluctuations in the fMRI blood oxygen level dependent (BOLD) signal in the absence of a specific stimulus. Many studies have identified differences in resting state 'networks' between subjects with a range of neurological and psychological disorders ranging from multiple sclerosis and Alzheimer's to schizophrenia and ADHD. However, this analysis assumes that all changes in network connectivity are differences at the neuronal level. In this work, we ask two questions: 1) Can all resting state hemodynamic fluctuations be interpreted as representing underlying neuronal activity? and 2) Do alterations in resting state functional connectivity always represent neuronal differences, or could they also represent imbalance in neurovascular coupling?
To address these questions, we acquired both multi-spectral optical intrinsic signal imaging (MS-OISI) of cortical hemodynamics and multi-location electrophysiology in a bilaterally exposed cortex rat model. Electrodes were located within the bilateral hind-paw regions of the somatosensory cortex. Simultaneous electrical and hemodynamic signals were acquired both during and in the absence of sensory stimulation. Both local field potential (LFP) and multi-unit activity were compared to the observed local and distant hemodynamic responses to determine whether similar models can predict both stimulus-evoked and spontaneous hemodynamic fluctuations. A detailed analysis of stimulus-evoked and resting-state neurovascular coupling will be presented.

Disclosures: Y. Ma: None. **A. Rayshubskiy:** None. **S.H. Kim:** None. **D. Timerman:** None. **M.G. Kozberg:** None. **E.M.C. Hillman:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.17/HHH39

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: GARFS8414

SF0180148s08

Title: Histochemical characterization of Wfs1 protein in mouse pituitary

Authors: *K. TEIN, A. TERASMAA, E. VASAR, S. KÕKS;
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Abstract: Mutations in WFS1 gene cause Wolfram syndrome, which is a rare autosomal recessive disorder, characterized by diabetes mellitus, diabetes insipidus, optic atrophy and deafness (DIDMOAD). WFS1 gene product wolframin is located in the endoplasmic reticulum and mouse lacking this gene have disturbances in peptide processing and secretion. High levels of wolframin protein are observed in the hippocampus, amygdala and limbic structures, but Wfs1 protein has not been studied in pituitary gland. It is important to know localization of Wfs1 protein in pituitary to understand better the regulation of pituitary peptides and hormones. Proopiomelanocortin (POMC) is a precursor polypeptide and is located in the anterior lobe and in the intermediate lobe of the pituitary gland.
Prohormone convertase (PC2) is located in neurons and endocrine cells and is needed for processing the precursors of neuropeptides and peptide hormones, such as vasopressin and POMC. Maturation and activity of PC2 depends on neuropeptide secretogranin V (7B2).
In this study, we used immunohistochemistry (IHC) to characterize Wfs1 protein localization in mouse pituitary. As Wfs1 takes part in protein processing, we also used IHC to detect possible co-localization of Wfs1 and POMC. Moreover, co-localization of PC2 and 7B2 with POMC was detected.
We found that Wfs1 is localized in anterior pituitary and apparently is not co- localized with POMC. In intermediate pituitary, PC2 and POMC are co-localized, while PC2 cannot be found in anterior nor posterior pituitary. 7B2 and POMC are also co- localized in intermediate pituitary. Immunohistochemistry also showed that 7B2 can be found in anterior pituitary and in posterior pituitary, though 7B2 and POMC are not co- localized in anterior pituitary.
Current results suggest that Wfs1 is expressed in anterior pituitary with POMC, notably PC2 seem to be absent in this part. Therefore, it is possible, that Wfs1 takes part in processing of POMC in absence of PC2. Further studies are needed, to characterize possible co-localization of Wfs1 with other neuropeptides in pituitary. Such study would suggest the identity of neuropeptides, which processing is regulated by Wfs1.

Disclosures: K. Tein: None. **A. Terasmaa:** None. **E. Vasar:** None. **S. Kõks:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.18/HHH40

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NSF CBET-1264948

Wellcome Trust

Title: Convection-enhanced delivery based staining method and simultaneous multi-wavelength optical imaging of calcium and intrinsic signals

Authors: *H. MA[1], S. HARRIS[2], M. ZHAO[1], J. BERWICK[2], T. H. SCHWARTZ[1];
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Abstract: Neurovascular coupling is the fundamental of many functional brain imaging techniques, such as functional magnetic resonance imaging (fMRI). The clinical application of these imaging techniques relies on this coupling to infer the spatiotemporal neural dynamics. An understanding of these coupling, especially spatial coupling, remains largely unclear because a method that can simultaneously record hemodynamic can neuronal activity with same spatiotemporal resolution is missing. We used convection-enhanced delivery to stain the whole hemisphere with calcium dye and perform simultaneous multi-wavelength optical imaging to record neuronal and hemodynamic activities with same spatiotemporal resolution. The fast component of calcium imaging data can offer similar information as voltage sensitive dye imaging without occupying the popular wavelengths used in hemodynamic imaging. The slow component can reflect the activity of glia cells. We have demonstrated that this technique is useful in study neurovascular coupling during different brain activities, such as spontaneous activity, sensory processing and pathology condition like epilepsy.

Disclosures: H. Ma: None. **S. Harris:** None. **J. Berwick:** None. **M. Zhao:** None. **T.H. Schwartz:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Support: R01 NS076628 (NINDS)

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UL1 RR024156 (NCATS)

NSF 0954796

Human Frontier Science Program (HFSP)

Title: Longitudinal assessment of resting state functional connectivity variance in the rat brain

Authors: *S. H. KIM, V. VOLETI, Y. MA, E. RAMIREZ, M. G. KOZBERG, M. B. BOUCHARD, B. R. CHEN, A. RAYSHUBSKIY, E. M. C. HILLMAN;
Biomed. Engin., Columbia Univ. Lab. For Functional Optical Imaging, New York, NY

Abstract: Resting state functional connectivity mapping assesses correlations between spontaneous fluctuations in the functional magnetic resonance imaging (fMRI) to image blood oxygen level-dependent (BOLD) signal throughout the brain. Correlated regions are interpreted as belonging to the same functional network. fMRI BOLD signals are derived from changes in deoxyhemoglobin concentrations, and therefore represent hemodynamic and not neuronal fluctuations. As a result, although hemodynamic activity in the brain is generally well-coupled to neuronal activity, it is important to assess whether functional connectivity results truly represent a physical property of neuronal connectivity and communication in the brain. To address this, we assessed resting state hemodynamics and their bilateral correlations in a chronic rat model using multispectral optical intrinsic signal imaging (MS-OISI). After wide-field aseptic thinned-skull craniotomy, animals were imaged up to 6 times over the course of 2 weeks. In each imaging session, the animal was anesthetized with either ketamine or varying doses of isoflurane, with terminal experiments performed under urethane anesthesia. We hypothesized that if functional connectivity networks derived from all anesthesia conditions were identical, that results represented a true functional network within the brain of a given animal. Systematic, anesthesia-independent modifications to functional connectivity maps would represent adaptation of the animal and its brain to the craniotomy. Instead, our results to date demonstrate that different anesthesias invoke highly varying resting state hemodynamics, in terms of spectral frequencies and bilateral spatial coherence. Resting state functional connectivity networks were therefore most strongly correlated to anesthesia type and level, rather than to each rat's innate 'network'. In ongoing work, we are extending these studies to incorporate indwelling electrodes and imaging without anesthesia to determine whether the influence of each anesthesia on the

representation of functional mapping is the result of alterations in neuronal or hemodynamic (neurovascular) control under anesthesia. These results are important for understanding and interpreting differences in resting state networks that have been observed in different pathological states, since the condition of the brain, and the integrity of neurovascular coupling in those conditions could underlie alterations in networks otherwise assumed to be wholly neuronal.

Disclosures: S.H. Kim: A. Employment/Salary (full or part-time):; Paid Research Assistant. **V. Voleti:** None. **Y. Ma:** None. **E. Ramirez:** A. Employment/Salary (full or part-time):; Paid Research Assistant. **M.G. Kozberg:** None. **M.B. Bouchard:** None. **B.R. Chen:** None. **A. Rayshubskiy:** A. Employment/Salary (full or part-time):; Paid Research Assistant. **E.M.C. Hillman:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 282.20/HHH42

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: American Heart Association Grant 0930132N

NIH Grant P30NS052519

Title: Mitochondrial Ca2+ uptake capacity impacts both intrinsic and evoked brain activities

Authors: *S. S. KANNURPATTI[1], B. G. SANGANAHALLI[2], P. HERMAN[2], F. HYDER[2]; [1]Radiology, Umdnj-New Jersey Med. Sch., Newark, NJ; [2]Diagnos. Radiology, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Despite vast knowledge on the cellular mechanisms of Ca2+ integration by mitochondria [1] its in vivo impact in the brain is not known. Mitochondrial Ca2+ uptake can regulate neural signaling [2] and oxidative energy metabolism [3], both of which are important to understand the biophysical basis of functional magnetic resonance imaging (fMRI). fMRI is increasingly used in clinical neuroscience to determine physiological and pathological brain functions in humans. Therefore we studied the in vivo mitochondrial functional impact on the intrinsic and evoked activity states of the brain by investigating cerebral blood flow (CBF), neural activity and fMRI blood oxygen level dependent (BOLD) measures in an integrated manner using both resting and task-related designs. In vivo mitochondrial function was pharmacologically modulated by altering mitochondrial Ca2+ uptake capacities by treating α-

chloralose or urethane-anesthetized rats with Ru360 or Kaempferol which inhibits or enhances the mitochondrial calcium uniporter (mCU) respectively. Inhibition of mitochondrial Ca2+ uptake by Ru360 reduced the spontaneous and evoked neural activities whereas enhancement of mitochondrial uptake by Kaempferol increased both. Furthermore the way neuronal activity coupled to CBF and BOLD responses was dependent on mitochondrial Ca2+ uptake capacity with a relatively lesser impact on the CBF hemodynamic responses compared to BOLD. The different neural-CBF and neural-BOLD relationships during the altered mitochondrial states suggested that mitochondrial Ca2+ uptake capacity impacted oxidative energy metabolism-related blood oxygen signals more intensely during both intrinsic and evoked activity conditions. Depressed mitochondrial functions occur in aging, Alzheimer's and Parkinson's disease [4,5] whereas in some other neuropathologies such as pediatric epilepsy hyperactive mitochondrial functions occur [6,7]. The results collectively suggest that mitochondrial Ca2+ handling in vivo need to be considered from relevant preclinical animal models to better interpret intrinsic and evoked brain activity differences in human neuropathologies.

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Disclosures: S.S. Kannurpatti: None. **B.G. Sanganahalli:** None. **P. Herman:** None. **F. Hyder:** None.

Poster

282. **Blood Flow and Blood Brain Barrier**

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Program#/Poster#: 282.21/HHH43

Topic: G.04. Physiological Methods

Support: NSC 101-2811-B-182-029

NSC 101-2321-B-182-012

Title: The brain modulatory effect of focused ultrasound-induced blood-brain barrier disruption

Authors: *H.-Y. LAI[1,2], P.-C. CHU[3], H.-C. TSAI[4], K.-Y. CHANG[5], Y.-Y. SHIH[6], H.-L. LIU[3], Y.-Y. CHEN[7], Y.-C. PEI[1,2];
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Abstract: The blood-brain barrier (BBB) protects neural tissues from the entry of toxins and infectious materials and also prevents most therapeutic agents passing into the brain. Focused ultrasound (FUS) co-administrated with intravenously circulating microbubbles can disrupt BBB. However, our recent study found that FUS with microbubbles affects the brain activity evidenced by a transient decrease of somatosensory evoked potential (SSEP) even using a relatively low acoustic pressure of 0.3 MPa. In the present study, we characterized the change of blood oxygen level dependent (BOLD) to investigate the correlation between FUS-induced changes of hemodynamic responses and neuronal activities. We hypothesized that the coupling between hemodynamic responses and electrical activities remains even after the administration of FUS. The present study also investigated the time course of functional recovery by observing the changes of BOLD responses and SSEPs in varying FUS acoustic pressures.
To this end, we injected SonoVue SF6-coated microbubbles (2-5 μm diameter) intravenously and transmitted FUS with acoustic pressures of 0.2, 0.3, or 0.4 MPa and recorded BOLD responses and SSEPs elicited by forepaw electrical stimulation before and after FUS to observe the time course of suppression induced by FUS. The FUS (burst duration=10 ms, pulse-repetition frequency=1 Hz) was delivered to the left S1FL cortex (1 mm posterior and 4 mm lateral to the bregma) to 40 SD rats weighing 300-350 g (n=20 for BOLD, n=20 for SSEP) under 0.1 mg/kg Domitor anesthesia. BOLD responses were obtained from a Bruker 7T system using a gradient-echo EPI sequence (BW=200 kHz, TR/TE=2000/20 ms, Matrix=80x80, FOV=2.5x2.5 cm^2, slice thickness=1 mm). The SSEP was recorded by epidural electrodes implanted on the scalp. The stimulation paradigm was a regular bipolar square-wave current of 6 mA with a pulse width of 0.2 ms and a frequency of 3 Hz.
The 0.4-MPa FUS exposure immediately suppressed both BOLD responses and SSEPs and the effect could last for 7 days ($p < 0.05$). The 0.3-MPa FUS exposure suppressed BOLD responses and SSEPs with a smaller magnitude compared to that induced by 0.4-MPa FUS exposure, and the suppression gradually weakened and disappeared 3 days following FUS. The 0.2-MPa FUS exposure did not suppress BOLD responses or SSEPs, and did not open the BBB, neither. The present study demonstrated the correlation of the change of BOLD responses and SSEP signals following FUS-induced BBB disruption, indicating the preservation of neurovascular coupling

that implies the potential utility of fMRI for assessing the brain modulatory effects induced by FUS.

Disclosures: H. Lai: None. **P. Chu:** None. **H. Tsai:** None. **Y. Shih:** None. **H. Liu:** None. **Y. Chen:** None. **Y. Pei:** None. **K. Chang:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 283.01/HHH44

Topic: F.01. Human Cognition and Behavior

Support: UConn Large Faculty Grant

Title: Conditioned place preferences in hungry and non-hungry humans

Authors: *R. S. ASTUR, A. W. CAREW, B. E. DEATON;
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Abstract: Research has shown that nonhumans can be conditioned to prefer a neutral environment by pairing that environment with a rewarding stimulus, whether it is food, water, drugs, or social interactions. In an attempt to extend this paradigm to humans, we created a virtual reality (VR) conditioned place preference task, with real-life rewards. In addition, we examined how hunger affects the strength of conditioning.
In Experiment 1, 21 undergraduates were recruited and food restricted by asking them not to eat between midnight and 9am for two consecutive days. In Experiment 2, 21 additional undergraduates were recruited and allowed to eat normally. This was a 2-day study: Day 1 was a pairing/conditioning day, and Day 2 was the place preference test day. Participants were placed into a VR environment consisting of 2 visually distinct rooms connected by a hallway. On Day 1, participants underwent 6 pairing sessions in which they were locked into one of the two rooms and were to explore the VR environment by using a joystick to move throughout the VR world. Room A was paired with real-life M&Ms for 3 sessions, and Room B was paired with no food for 3 sessions. During the M&M sessions, chocolate M&Ms were periodically dispensed into a cup and the participant was instructed to eat the M&Ms as they were dispensed; approximately 50-60 M&Ms were administered on Day 1. Room / M&M pairings and ordering were counterbalanced. Day 2 was the test day, administered the next day, and participants were given free access to the entire VR environment for 5 min.
For the hungry participants, we observe that participants spent 49% of the time in the room previously paired with M&Ms compared to 21% in the room paired with no food. Hence,

participants display a significant conditioned place preference for a VR room that was previously paired with food ($p < 0.001$). Additionally, they display a significant explicit preference for the M&M-paired room. However, for the non-hungry participants, there was no evidence of a place preference, either implicitly (e.g. dwell time) or explicitly. Hence, we show that we can reliably establish a place preference in humans, but that the preference is contingent on the participants' hunger state. Future research will examine the extent to which these preferences can be blocked or extinguished as well as whether these preferences are evident using other reinforcers.

Disclosures: R.S. Astur: None. **A.W. Carew:** None. **B.E. Deaton:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 283.02/HHH45

Topic: F.01. Human Cognition and Behavior

Title: Skill acquisition in piano performance: The influence of flow on learning

Authors: *Y. MIYAKE[1], H. YANO[1], A. NAKAMURA[1], K. KATAHIRA[1], S. FURUYA[2], N. NAGATA[1];
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Abstract: In various fields, such as the arts, sports, and design, the elucidation of the process of skilled movement acquisition is an important topic of research. We focused on the process of piano performance skill acquisition and examined the effect of intrinsic motivation as a factor in promoting the learning effect in particular. The present longitudinal study aimed to investigate the relationship between acquisition of performance skills and flow as an index of intrinsic motivation. Twenty-four right-handed adult participants participated in an hour of piano practice twenty times. Each participant selected a favorite piece from among several musical pieces and practiced it. At the end of each practice, participants answered a questionnaire about the flow state experienced during the practice. To determine the effects of training, several tests to assess fundamental hand motor functions were carried out before and after the twenty hours of practice. The tests were of three types; one consisted of playing a short tone sequence of twelve strokes as fast as possible (speed test), another consisted of playing a tone sequence synchronized with a metronome (accuracy test), and the third consisted of tapping fingers except the thumb as fast as possible (tapping test). In order to assess the result of the tests, the timing and velocity information for each keystroke was collected from an electric piano. The results of the flow

experience questionnaire were analyzed by cluster analysis and were divided three groups on the basis of tendency toward shift of flow over twenty hours; the first group maintained a high level of flow for twenty hours (A group), the second had a high level of flow at first and a declining level of flow toward the end (B group), and the third had a low level of flow constantly (C group). Among these three groups, we compared the improvement of hand motor functions through practice. Improvement of hand motor functions was observed before and after practice for the A and B groups, which had a high level of flow. A difference was observed between the results of the speed test before and after practice in these two groups; they acquired ability to play the tone sequence faster. In the tapping test, differences were observed between the results before and after practice only for the A group; they improved independence of movement across fingers. In the accuracy test, the accuracy of performance was improved only for the B group. The C group, which had a low level of flow, showed no improvement of hand motor functions in any tests. Therefore, the specific state in which flow was experienced or the flow experience itself was suggested to be effective in learning of hand motor functions.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Numbers 23700137

JSPS KAKENHI Grant Numbers 23300216

Title: Neural basis of cognitive and motivational dynamics relevant to sustainable human-agent interaction: An fMRI study

Authors: *T. NOZAWA[1], Y. SASAKI[1], R. YOKOYAMA[1], Y. AKIMOTO[1], R. NOUCHI[1], M. SUGIURA[1], T. KONDO[2], R. KAWASHIMA[1];
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Abstract: Human-agent interaction (HAI) involves virtual or real agents and has a wide range of applications. Development and sustenance of motivation for HAI with an unknown agent is affected by various cognitive and emotional dynamics, such as prediction, adaptation and exploration, and frustration. This fMRI study investigated neural substrates of these dynamical

processes. Three runs of fMRI scan were conducted on 27 university students while they engaged in HAI with three types of agents with different predictability. A virtual agent was presented in the form of a dog-type robot. Subjects pressed one of three buttons, and the agent responded with one of three gestures. This sequential HAI continued for 16 min each session. The three types of agents were (a) a nonadaptive agent with random actions; (b) an adaptive agent with fixed input-reward mapping; and (c) an intrinsically motivated adaptive agent that pursued an intermediate level of novelty. The order of the agent types was counterbalanced. Subjects were not informed about the nature of the agents and were instructed to freely set and change their aims. Subjects were supposed to have a probabilistic internal model of their relationship with the agent, which would be updated using the HAI history. Applying information-theoretic formulation to the model, we estimated the time courses of three motivation-related internal state variables, as follows. (1) Intrinsic reward was high when the agent's reaction was highly predictable from the subject's action choice. (2) Intention to explore was high when selecting actions that were supposedly unexpected for the agent. (3) Contextual controllability expressed how the situation was controllable on average. These estimates were used as first-order parametric modulators of the input and output events in the individual-level SPM analysis over the all sessions, and the contrast images produced were subjected to group-level random effects analysis. This analysis showed that (1) activity in the thalamus was positively correlated with the intrinsic reward; (2) activities in the left frontal eye field and bilateral intraparietal sulcus were positively correlated with the intention to explore; and (3) activities in the bilateral insula cortex were negatively correlated with the contextual controllability. The results can be interpreted as the neural substrates for (1) encoding an action-specific value signal; (2) exploratory decision-making; and (3) frustration when the situation is out of control, respectively. In conclusion, the model-based analysis successfully identified the neural basis of the cognitive and motivational dynamics relevant to sustainable HAI.

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Poster

283. **Human Learning: Reinforcement and Reward**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 283.04/III1

Topic: F.01. Human Cognition and Behavior

Support: AFOSR Grant FA9550-12-1-0355

Title: A computational cognitive neuroscience model of complex skill learning

Authors: *V. V. VALENTIN[1], W. T. MADDOX[2], R. MUSGRAVE[1], F. G. ASHBY[1];
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Abstract: Procedural learning of complex skills requires dopamine mediated striatal plasticity. Most prior work investigated single stimulus-response learning followed by feedback. However, many skills are composed of several actions that must be performed in sequence before feedback is available (e.g., as when a pilot executes a banked turn). We developed a category-learning task in which highly discriminable fractal images were presented sequentially, each requiring an A or B category response, but only the third response was followed by aggregate feedback. Correct feedback was given if all three responses were correct, and error feedback was given if any of the three responses were incorrect. Learning occurred in all three positions, although the best learning was to the stimulus in position 3. We propose and test a neurobiologically detailed theory of a dopamine dependent, unstructured category-learning network using aggregate feedback. The theory assumes that the category learning is mediated by plasticity at cortical-striatal synapses, which are modified by dopamine-mediated reinforcement learning. A model-based temporal difference learning algorithm propagates the dopamine neuron response back from the reward to the three categorization stimuli. The model successfully accounts for many properties of the human behavioral data.

Disclosures: V.V. Valentin: None. **F.G. Ashby:** None. **R. Musgrave:** None. **W.T. Maddox:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: DoD Grant W81XWH-09-2-0044

NIMH/FIC Grant R21MH095656

Title: Post traumatic stress disorder (PTSD) impairs reward learning on a probabilistic category task

Authors: *M. M. HERZALLAH[1,2], A. D. BROWN[3], D. ABU-AMARA[3], J. SCOTT[3], K. THACHER[3], C. R. MARMAR[3], M. A. GLUCK[1];
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Dis, Jerusalem, Palestinian Territory; [3]PTSD Res. Program, Dept. of Psychiatry, NYU, New York, NY

Abstract: Using a probabilistic category-learning task that allowed for dissociation between the acquisition of positive feedback (reward) and negative feedback (punishment) during learning, we tested US combat veterans who served in Iraq and Afghanistan with and without Post-Traumatic Stress Disorder (PTSD). Although individuals with and without PTSD learned equally well in response to negative feedback, individuals with PTSD exhibited minimal learning from reward stimuli. These results suggest that PTSD may be characterized, in part, by alterations in cortico-striatal pathways, and deficits in reward learning may reflect an important, but relatively unexplored mechanism underlying PTSD. Further research examining the neurobiology associated with deficits in reward learning in PTSD may serve as an important marker in assessment and treatment. Although considerable work has demonstrated the importance of alterations in the neural circuitry of fear in people with PTSD, other neural circuits, such as those for reward learning (which have received considerably less attention), may play a critical role in the pathogenesis of the disorder.

Disclosures: M.M. Herzallah: None. **A.D. Brown:** None. **D. Abu-Amara:** None. **J. Scott:** None. **K. Thacher:** None. **C.R. Marmar:** None. **M.A. Gluck:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Support: Grant from the Wellcome Trust

Title: Distinct neural computations mediating observational learning derived from facial expressions versus words

Authors: *D. A. STANLEY[1,2], J. STANLEY[2], R. M. PIECH[2,3], J. P. O'DOHERTY[2,1]; [1]Caltech, Pasadena, CA; [2]Trinity Col. Dublin, Dublin, Ireland; [3]Anglia Ruskin Univ., Cambridge, United Kingdom

Abstract: We often learn about the value of actions not through doing them ourselves, but through watching other people do them and observing the outcomes they experience. Much is known about the neural computations that support learning through experience, but relatively little about those that support vicarious observational learning. A major open question is how

does the source of information being observed influence observational learning: e.g. is learning through observing others' affective reactions different from gaining verbal feedback about outcomes they experienced? To address this question we investigated the neural computations of observational learning, conveying information about others' experienced outcomes using either facial expressions, words, or both.
fMRI data were collected from 25 observers. Observers learned about the likelihood that 6 lottery machines would deliver aversive (salty tea) or neutral (artificial saliva) outcomes by watching videos of 3 observees play the lotteries. Each observee was associated with a pair of lotteries and 1 of 3 interleaved conditions. The conditions were: 1) Face Only (FO), observers saw videos of observees receiving outcomes paired with a nonsense word. 2) Word Only (WO), observers saw videos of observees in which their face conveyed no expressive feedback, paired with words indicating the experienced outcome. 3) Face+Word (FW), observers saw both expressions and words depicting the experienced outcome. Lottery pairs contained one aversive (paversive=0.8, pneutral=0.2) and one neutral (paversive=0.2, pneutral=0.8) lottery. Lottery pair contingencies reversed randomly once during the experiment. To assess learning, observers completed randomly interspersed choice trials in which they chose between lottery pairs. Choice feedback was delivered between runs and without choice information.
Behavioral data were fit with a computational reinforcement learning model. Accuracy was above chance and did not differ across conditions, though learning rates were higher in the FW (0.51) than in the FO (0.37) and WO (0.38) conditions. fMRI analysis revealed signals in the amygdala and face-selective temporal/occipital cortex correlated with aversive prediction error (i.e. highest for unexpected aversive outcomes) in conditions where face expressions depicted outcomes (FO & FW). Conversely, fMRI signal in part of the caudate head correlated with appetitive prediction error in conditions in which words described outcomes (WO & FW). These data suggest that learning through observation on the basis of social and non-social observational feedback may depend on at least partly distinct neural substrates.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Support: NIMH Brains Award 203-1217

Title: Behavioral neurostimulation: Sustained activation of the human dopaminergic midbrain using real-time fMRI

Authors: *K. C. DICKERSON, J. J. MACINNES, R. A. ADCOCK;
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Abstract: Dopamine is critically important for human motivation, learning, and memory. Nearly all prior human research of the dopaminergic midbrain has used extrinsic cues to elicit changes in midbrain activation. We reasoned that humans could learn to use internal mnemonic representations to produce sustained activation within the dopaminergic midbrain; we refer to this as 'behavioral neurostimulation.' If behavioral neurostimulation of the dopaminergic midbrain were achievable, it would have putative applications for a) enhancing motivation, learning, and memory in healthy humans and b) translating this ability to clinical populations with aberrant dopamine functioning (e.g., patients with depression). The purpose of this experiment was to investigate if healthy individuals can learn to produce sustained activation of the dopaminergic midbrain, specifically the ventral tegmental area (VTA). We hypothesized a) that individuals can learn to produce sustained activation of the VTA and b) that real-time fMRI (rt-fMRI) neurofeedback will facilitate learning to produce sustained VTA activation. Thirty-nine healthy adults participated in the experiment, and were randomly assigned to either the Experimental or Control condition. Both groups were instructed to try to increase the BOLD signal within the VTA by using motivational thoughts/imagery. Both groups completed a pre-test run, 3 training runs, and a post-test run. During both test runs, participants tried to increase VTA BOLD signal without any rt-fMRI neurofeedback indicating their success. In the training runs, the Experimental group received rt-fMRI neurofeedback indicating the magnitude of BOLD signal in the VTA via a thermometer display. This level was updated every 1 second as each new incoming data point was acquired. The Control group viewed a similar thermometer display, which showed a repeating pattern indicating the period during which they should enhance their motivation, but the pattern did not contain information about the activation of any brain region. Results revealed that the Experimental group successfully produced enhanced, sustained activation in the VTA. Furthermore, within the Experimental group, individuals who were initially not good at VTA self-activation improved significantly with training, whereas those who were already good at VTA self-activation did not change significantly over time. This is the first demonstration of enhanced, sustained VTA activation in humans, and suggests that behavioral neurostimulation is a feasible therapeutic modality.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Support: National Institute for Information and Communications Technology, Japan

Wellcome Trust

Title: Dissociating specific and general conditioned responses in Pavlovian aversive conditioning

Authors: *S. ZHANG[1], H. MANO[1], G. GOWRISHANKAR[1], T. ROBBINS[2], B. SEYMOUR[3,1,2];
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Abstract: Understanding the mechanisms of Pavlovian (Classical) conditioning is a central concern for contemporary studies of emotion, memory and decision-making. Psychological and physiological studies rely on the ability to demonstrate conditioned responses: these are elicited by the conditioned stimulus (cue) and reflect the prediction of a salient aversive or rewarding outcome. Classical psychological and ethological descriptions of conditioned responses divide them into stimulus general responses, which are common to all outcomes of the same valence (e.g. withdrawal to aversive events), and stimulus specific responses, which reflect the particular nature of the outcome (such as leg flexion to foot shock). However, it is not known to what extent these two mechanisms can be functionally dissociated, and whether specific conditioned responses can be clearly lateralized in humans. Here, we will present preliminary results from two behavioural studies of aversive conditioning, in which we try to dissociate laterality-specific limb electromyographic changes. We implemented a first order probabilistic delay conditioning design, in which visual cues were followed by thermal pain stimuli to either right or left hand. We established contingencies between different cues that 1) reciprocally and 2) independently predicted high probability pain to each hand. We recorded limb EMG, facial EMG, and autonomic responses. Preliminary results support the existence of dissociable specific and general conditioned responses: we show that limb EMG responses clear reflect the specific prediction of pain to the side being presented, with a progressive development of the responses over time, which can be modeled using a time-rich temporal difference model. These are clearly dissociable from general autonomic responses. Our results support the existence of dissociable subprocesses underlying aversive learning.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

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Title: The role of adaptive decision noise in exploration

Authors: *R. C. WILSON[1], J. M. WHITE[2], J. D. COHEN[1,2];
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Abstract: Everyone at the SfN conference faces the explore-exploit dilemma: do you go see posters from labs you know well (exploit) or wander aimlessly down row BBB in search of something new (explore)? Exploiting is the best way to get immediate reward, but if you don't explore you might miss out on the latest advances. Solving this problem optimally is intractable in all but the simplest settings, and so the question arises as to how humans balance exploration and exploitation in practice.

In machine learning, engineers have made use of noise as a tool for driving exploration. This strategy works by injecting randomness into the decision process. When little is known and learning is desired, noise is increased, promoting exploration. However, as learning occurs, and the reward structure is better understood, noise is decreased in order to exploit known sources of reward. Thus, the level of decision noise regulates the degree to which exploration or exploitation is favored.

We have previously shown that, in a very simple problem, humans adapt their decision noise in a way that is consistent with such a random-exploration strategy [1]. In the present work we tested whether this was also true in a more complicated experiment in which the potential gains for exploring were higher. In particular, we investigated the explore-exploit tradeoff in a world characterized by abrupt and unsignaled change-points. By manipulating the frequency with which change-points occurred, we were able to alter the optimal balance between exploration and exploitation and hence the optimal setting of the noise.

In our change-point task, participants made a series of choices between two options. Every time an option was chosen it paid out a reward between 0 and 100 points. The reward from each option was constant over time except at a change-point, when it was randomly reset between 0 and 100. Because the current reward value of each option was only shown when that option was

played, the longer an option remained unplayed, the more ambiguous it became, as the probability that a change-point had occurred increased.
We modeled human decisions using a simple choice rule based on the observed outcome for each option, the information available for playing it and the level of decision noise. As change-points became more frequent we found systematic increases in the decision noise. A separate analysis showed that this qualitative pattern of increasing decision noise with change-point frequency is optimal in this task.
These results suggest that humans both use and adapt their decision noise to effectively manage the explore-exploit tradeoff in complex tasks.
[1] Wilson et al. Program No. 830.13. Society for Neuroscience, 2011

Disclosures: R.C. Wilson: None. **J.M. White:** None. **J.D. Cohen:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust Grant

Niels Stensen Stipendium

Title: Adjustment to variability in a reward learning task is beneficial for performance

Authors: *K. M. DIEDEREN[1], T. J. SPENCER[2], P. C. FLETCHER[2], W. SCHULTZ[1];
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Abstract: An essential part of daily life is to predict which rewards will be available. We might achieve sufficient accuracy in this by adjusting our expectations to a number of factors including the variability of the environment. In addition, we frequently move between environments in which we retain our beliefs after switching to a new situation. Indeed, previous studies have shown that individuals efficiently update their predictions in noisy and volatile situations by assigning more weight to certain outcomes. Importantly, this can be determined by measuring participants' predictions and learning rates (LR). Here we investigated if individuals could adjust to variability in a reward learning task in which we alternated different levels of variability in short blocks. In addition, we tested if individuals reached similar levels of accuracy under different levels of variability and if this was predictive of overall accuracy. Finally, as variance is

often undervalued by sensation seekers we tested if subjects' level of sensation seeking was predictive of performance. We used a task in which 29 participants predicted reward magnitude on a trial by trial basis. Reward magnitudes were drawn from pseudo-Gaussian distributions with three different standard deviations (SD) which alternated in blocks of 5-8 trials. Following their prediction, participants received a reward which elicited a prediction error (PE) and enabled them to adjust their upcoming predictions. LR was derived by calculating the fraction of the PE on trial *t* that was used to update the prediction on trial *t+1*. The speed of learning (number of trials to asymptote) was faster when variability of the reward distribution was smaller. This was due to participants having higher learning rates for smaller SDs before asymptote was reached. Only the speed of learning was affected by the level of variability as participants reached similar levels of accuracy (difference from the expected value (EV)) for the different standard deviations. As a result, PEs increased monotonically with the level of variability, a difference that disappeared after normalising PEs by the SD of each distribution. Furthermore, participants reached very high accuracy (predictions close to the EV) for all SDs, indicating that switching between conditions did not impede learning. Importantly, participants that had a similar accuracy for the different SDs i.e., those that appeared to adapt best to changing variability, showed a higher level of overall accuracy. Finally, this was related to individuals' tendency towards sensation seeking with subjects' scoring higher on this trait showing superior adjustment to variability.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Title: Stress and pressure: Differential effects on physiological reactivity and cognitive performance

Authors: *S. B. HUTCHINSON[1], S. K. MCCOY[1], L. HAWTHORNE[1], S. W. ELL[1,2]; [1]Psychology, Univ. of Maine - Psychology Dept., Orono, ME; [2]Grad. Sch. of Biomed. Sci., Univ. of Maine, Orono, ME

Abstract: From family life to social life to work life, pressure and stress are so ubiquitous in modern life that it is no surprise that cognitive neuroscientists have taken great interest in the

impact of pressure and stress on cognitive performance. Recent data suggest that the impact of stress hormones on prefrontal cortical function may be a key mediator of this complex relationship. Across a series of experiments, we investigated the impact of pressure and stress on rule-based (learning is mediated by a hypothesis-testing system that is dependent upon prefrontal cortex) and information-integration (learning is mediated by a procedural-based system that is not dependent upon prefrontal cortex) category learning tasks. In Experiment 1, outcome pressure (i.e., pressure related to successful task performance) impaired accuracy on a rule-based task), had no effect on an information-integration task, but was not physiologically stressful. In Experiment 2, the addition of monitoring pressure (i.e., pressure related to social evaluation) impaired performance on both categorization tasks. Outcome + monitoring pressure was moderately physiologically stressful, but stress reactivity was unrelated to categorization performance. We compare the data from Experiments 1 and 2 to a previous study from our lab investigating the impact of a social-evaluative stressor on categorization performance. In contrast to the pressure manipulations, a social-evaluative stressor led to a robust stress response that was predictive of impaired rule-based performance and enhanced information-integration performance. In sum, in order to understand the relationship between stress, pressure, and cognition, it may be necessary to consider variability in the pressure situation, variability in the stress response, and variability in the cognitive and neural systems mediating task performance.

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Poster

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JST.CREST

Title: Neural computations mediating one-shot learning in the human brain

Authors: *S. LEE, J. P. O'DOHERTY, S. SHIMOJO;
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Abstract: In standard associative learning, an animal typically must incrementally experience a number of pairings between a stimulus and a consequence before a given stimulus pairing is fully learned. However, animals sometimes encounter outcomes that they have never experienced previously from which it is necessary to learn rapidly in order to survive. In such cases, animals can learn on the basis of only a single exposure to a stimulus pairing, a situation described in the literature as "one-shot" learning. For example, in taste aversion learning only a single exposure to a food accompanied with delayed sickness or nausea is sufficient for learning to occur. While the neural computations underlying incremental learning are gradually being elucidated, very little is known about how, at the computational level, one-shot learning might be implemented in the brain.

One-shot learning imposes a substantial challenge to standard learning algorithms including Bayesian models and power PC theory, as such models are not optimized to facilitate learning from a single experience. Here, we propose a novel computational mechanism for one-shot learning in which a Bayesian learner attempts to establish the casual relationship between stimuli and outcomes. Critically, this learner uses knowledge about the uncertainty with which a particular stimulus has caused a given outcome to drive the extent to which learning occurs about that stimulus. Stimuli paired with outcomes about which there is large causal uncertainty can result in very rapid learning, such that even within a single-trial substantial learning has occurred. We applied this model to fMRI and behavioral data from human participants while they engaged in a task designed to assess one-shot causal learning. Our fMRI data suggests that parts of the prefrontal cortex including the ventrolateral prefrontal cortex (vlPFC) were found to be involved in encoding causal uncertainty that was postulated by the model to drive changes in the learning rate needed for one-short learning. Crucially, functional coupling between the vlPFC and areas involved in episodic memory encoding were significantly increased during the period in which the model predicts one-shot learning compared to situations where incremental learning was taking place. These findings suggest that vlPFC may act as a switch: engaging episodic memory systems when learning needs to proceed from a single episode (one-shot) as opposed to incrementally. Taken together these findings form the basis of a new understanding of the neural computations underlying the ability to learn from a single exposure to an event and its consequences.

Disclosures: S. Lee: None. **J.P. O'Doherty:** None. **S. Shimojo:** None.

Poster

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Title: Brain activity by selfish partner dynamically modulates the reward-related cortical network

Authors: *M. SHIKAUCHI[1], H. MIZUHARA[2];
[1]Dep. of Dynamic Brain Imaging, Advanced Telecommunications Res. Inst. Intl. (ATR), Kansai Science City, Japan; [2]Kyoto Univ., Kyoto City, Japan

Abstract: An abstract item (e.g., money) activates the same reward-related regions in the human brain as do primitive rewards (e.g., food). These regions can also be activated by various stimuli, such as social stimulus/condition (e.g., admiration). The reward system needs to be dynamically modulated in order to adapt to fluid environments in the social environment because humans obtain their rewards during interactions with both cooperative and selfish partners.
Assuming that a partners' cooperative/selfish behavior would be a crucial factor in the modulation of the reward system and that this phenomenon would be produced by the interaction of social- and reward-related brain activity, we hypothesized that a partner's cooperativeness would modulate the reward-related activity and network. Recording brain activity by fMRI, we investigated this using as an experimental task a modified version of the centipede game. In this task, which is a dynamic version of the prisoner's dilemma, participants were required to decide their strategy according to their partner's cooperativeness.
Results showed that the caudate correlated significantly with the reward value using a general linear model analysis, and that the middle cingulate cortex (MCC) and putamen activated significantly in the case of a selfish partner. To investigate the relationship of these regions, we estimated effective connectivity by Dynamic Causal Modeling (DCM). This DCM analysis showed there to be an effective connectivity from the caudate to the putamen, and that this was modulated by the effect of the MCC activity so that the putamen activity was increased when the partner was selfish.
These results suggest that caudate-putamen connectivity as the reward system can be modulated by social-related activity. Our view would be also that the network modulation system recreates the variety of reward and flexibility found in the social environment.

Disclosures: M. Shikauchi: None. **H. Mizuhara:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Support: CIHR

NSERC

Title: Moving from model-free to model-based RL in a probabilistic learning environment: A generalized hierarchical reinforcement learning model

Authors: *M. BALCARRAS[1], S. ARDID[1], T. WOMELSDORF[2];
[2]Biol., [1]York Univ., Toronto, ON, Canada

Abstract: In real-world environments, learning optimal responses to stimuli faces a problem of scale: there is always a very large number of possible state to action-outcome mappings and many rewarding outcomes rely on correct complex action sequences. Structuring decision-making hierarchically is one of the solutions to the problem of scale because it facilitates the abstraction of state-action-outcome mappings from one situation to another (Botvinick, Cur Opin Neurob 2012). Here, we set out to test whether Hierarchical Reinforcement Learning (HRL) frameworks can be adapted to explain the shift between exploratory and exploitative behaviours in reinforcement learning during a probabilistic choice task.
We devised a probabilistic decision-making task with hidden reward structure where subjects were required to choose between two stimuli (each composed of two features: color and shape), with discrete trials grouped into blocks by stimulus sets and reward schedules. In half the blocks, different reward probability was associated with a particular stimulus feature (i.e. just shape or colour is predictive of reward), whereas in the other half of blocks, the likelihood of rewards were associated with combinations of features, i.e. colors and shapes, that formed an object. We observed that although a feature-reward block increases the scale of learning difficulty (3 shape and 3 colour combinations to consider as possibly predictive of reward), subjects deploy a policy of making choices in one domain of feature space, speeding learning of optimal responses. We also observed that subjects moved from periods of exploration into exploitation more rapidly during feature-reward blocks suggesting the deployment of a behavioural policy is rapid.
We compared several types of RL models to explain these behaviours. Model-free RL employs no learned information to structure state-action-outcome mappings, and was the best model for explaining behaviour in fixed-feature blocks. Model-based RL considers only a subset of possible choice options, employing previous experience to speed learning, and it was the best model for non-combined blocks. Neither model-free or model-based RL performs well overall and neither employs a mechanism to switch strategies. A generalized HRL model that employs

model-free learning to select a feature-based policy in only a single feature domain (model-based RL) captured subject behaviour best overall, suggesting that subjects initially explore the total space of stimulus-outcome mappings but when they detect feature related structure in a history of reward they deploy a behavioural policy that isolates one stimulus feature domain.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Title: What should you do when you don't know what to do: Balancing the tradeoff between reward exploitation and information extraction

Authors: *J. COCKBURN, M. J. FRANK;
Cognitive, Linguistic and Psychological Sci., Brown Univ., Providence, RI

Abstract: Learning and action selection are significantly complicated by the fact that observed events (e.g your flight departing on time) are often determined in part by unobserved processes (e.g weather conditions at your destination). The advantage of considering latent processes comes when events are predictable if considered in concert with their latent influences, but appear random otherwise. Knowing the hidden state is helpful not only for conditionalizing action selection, but also for conditionalizing learning to prevent interference about action-outcome associations across separable states (e.g it's the airline's fault and you should avoid them next time, or did bad weather impact everyone).
Considering latent processes imports additional levels of uncertainty about the true state of the latent process. Recent studies have reexamined choice behaviour through the lense of uncertainty, and have suggested that 'exploratory' behaviour can be conceptualized as targeting the reduction of various forms of uncertainty about action-outcome contingencies. However, very little work has examined whether agents actively select actions that would reduce uncertainty about the state of latent processes that determine those contingencies.
We propose a model that defines action selection in terms of both expected value and mutual information shared between action outcome and latent state. Critically, the tradeoff between reward exploitation and information extraction is a function of unexpected uncertainty (UC). In short, our model predicts that the probability of selecting informative actions increases as expected outcome violations mount.

We investigate this hypothesis using a latent structure reinforcement learning task. Participants were asked to repeatedly pick amongst a set of cards that could be drawn from one of two decks. Participants were never told which deck was in play, but were told that the deck in play would switch on occasion. They were also told that cards in both decks look alike, but could have different payoffs. Each deck included a card with high expected value (but low mutual information), and a card with high mutual information (but low expected value). Behavioural results reveal an exploitive strategy when UC is low, and an increased probability of foregoing possible reward to gather information when UC is high. This pattern was observed across a broad range of task parameters. It was even observed when the optimal exploitive strategy would be to pick the same card regardless of the current deck in play. These results suggest that UC predicts the prioritization of information relative to reward, and that the reduction of UC is valued in its own right.

Disclosures: J. Cockburn: None. **M.J. Frank:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 283.16/III13

Topic: F.01. Human Cognition and Behavior

Title: The reward neural network mediates optimistic belief updates

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Abstract: People tend to learn more from disconfirming information when it leads to favorable outcomes. The present study investigates the neural correlates of such optimistic belief updating in order to provide empirical evidence for a specific involvement of the reward neural network. 24 subjects estimated their likelihood of experiencing a total of 88 adverse life events while undergoing fMRI. After a first estimation, the official average probability of the respective event was presented, followed by a second estimation with the opportunity for an update. Based on a tendency to adopt the most rewarding future outlook, updates were expected to be higher when the average probability was desirable (lower than the first estimation), than undesirable (higher than the first estimation). Estimations were done either for oneself or for a similar other, resulting in a two-by-two design, with factors target (self vs. other) and valence (desirable vs. undesirable). Unbeknownst to participants, the average probability was experimentally

manipulated in order to systematically create the valence conditions.
Behavioral results confirmed that mean updates were higher for desirable than undesirable trials, and that this effect was stronger for self than other. FMRI analyses focused on neural activity that tracked increasing amounts of updates on a trial-by-trial basis (pFWE-corr<.05). During the second estimation, the activity in the medial orbitofrontal cortex (OFC) increased with increasing updates more strongly in desirable than undesirable trials, and within desirable trials more strongly for self than other. During the presentation of the average probability, the activity in an extended neural network including the insula, the ventral striatum and the anterior cingulate cortex predicted the subsequent amount of update to a greater extent in undesirable than desirable trials, and this effect was stronger for self than other.
While behavioral results show asymmetric updates that promote optimistic beliefs, fMRI results provide evidence for a motivational explanation of this bias, which is stronger for self-related judgments. The activity in the OFC associated with reward experience was higher, the greater the amount of desirable updates. However, in the moment of the confrontation with the disconfirming information, a greater 'learning signal' due to the prediction error should be necessary in order to learn from undesirable relative to desirable information. Exactly this was mirrored by a greater correlation of activity with the amount of subsequent updates in undesirable than desirable trials in regions known to encode for aversive prediction errors.

Disclosures: B. Kuzmanovic: None. **K. Vogeley:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

Location: Halls B-H

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Program#/Poster#: 283.17/III14

Topic: F.01. Human Cognition and Behavior

Support: NIDA Grant DA02812

Title: Conditioned responses to a drug cue in healthy, non-dependent humans

Authors: *L. M. MAYO, H. DE WIT;
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Abstract: In the treatment of drug addiction, one of the greatest challenges is propensity to relapse,even after long periods of drug abstinence. It is widely believed that conditioned responses, established between environmental stimuli and drugs, contribute to relapse. Studies with laboratory animals suggest that these associations develop through classical conditioning mechanisms, but studies with humans typically assess responses to cues that are assumed to have

been associated with drug, making it difficult to determine whether they reflect classical conditioning or other learning processes. Here, we used classical conditioning procedures in a controlled environment to study the process of acquisition of drug-conditioning in humans. Healthy, non-dependent human volunteers (N=30) completed a pre-test session in which we measured responses to two neutral stimuli (preference, subjective liking, attention, and emotional reactivity). Subjects then underwent 4 conditioning sessions; 2 with placebo and 2 with 20mg methamphetamine (MA; oral), in which one stimulus was always present when they received the drug and the other was present during placebo sessions. Then we re-assessed responses to the stimuli after conditioning. After conditioning, subjects exhibited an increase in preference for the drug-paired stimulus, as measured with a forced choice task, and an increase in subjective liking of the drug-paired stimulus. In addition, emotional reactivity to the drug-paired stimulus increased, as measured by facial electromyography recordings of zygomatic and corrugator muscles. Finally, subjects also demonstrated a bias in attention towards the drug-associated stimulus. These studies indicate that healthy volunteers develop conditioned motivational responses to drug-paired cues, under carefully controlled conditions, which are detectable with subjective and objective measures of emotional reactivity and attention. Future research will focus on individual variation in the acquisition and expression of the responses, neurobiological underpinnings, and pharmacological and behavioral methods to manipulate such responses.

Disclosures: L.M. Mayo: None. **H. de Wit:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

Location: Halls B-H

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Program#/Poster#: 283.18/III15

Topic: F.01. Human Cognition and Behavior

Support: NSF Career Development

Title: Intrinsic connectivity within and between large-scale functional brain networks supports learning

Authors: *R. T. GERRATY[1], J. Y. DAVIDOW[1], I. KAHN[2], D. SHOHAMY[1];
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Abstract: Learning is essential for adapting behavior to a changing environment. Research on the neural bases of learning and memory has indicated that there are multiple specialized learning systems that differ in both their cognitive functions and their neural substrates. Emerging data suggest that these systems operate not as separate modules but as interactive

networks, raising questions about the nature of these interactions and their behavioral implications. In this study, we focused on two central questions: First, to what extent does baseline connectivity between the striatum and the hippocampus relate to habitual vs. flexible learning at the behavioral level? Second, what role do interactions between these systems and broader large-scale functional networks play in learning? To address these questions, we characterized resting-state functional connectivity (rsFC) both within and between the striatum, hippocampus, and large-scale networks, and examined how these circuit-level interactions relate to behavior. We specifically focused on feedback-based learning and learning-guided decision making. Healthy participants were scanned with fMRI during rest, after having previously completed behavioral paradigms that involved feedback-driven learning as well as generalization of learned information to new decisions. Spatial Independent Component Analysis (sICA) was used as a model-free means of defining regions and networks of interest. Results revealed intrinsic connectivity within a robust network including the hippocampus, the ventral striatum, and the ventral medial prefrontal cortex during rest. Across participants, the strength of connectivity both within this network, as well as between these regions and other resting state networks, was related to behavioral measures of flexible learning and decision making, tested separately. These findings shed light on the nature of the interaction between the striatum and the hippocampus and their participation in a broader circuit that supports learning and decision making.

Disclosures: R.T. Gerraty: None. **J.Y. Davidow:** None. **I. Kahn:** None. **D. Shohamy:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: Wellcomet Trust 089589/Z/09/Z

Wellcome Trust and Medical Research Council G00001354

Title: The neural correlates of aberrant emotional learning and flexibility in OCD

Authors: *A. M. APERGIS-SCHOUTE[1,2], C. M. GILLAN[2,3], J. A. BLAY[1], E. FERNANDEZ-EGEA[1,2], N. A. FINEBERG[4], T. W. ROBBINS[2,3], B. J. SAHAKIAN[1,2];
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Abstract: Obsessive-compulsive disorder (OCD) is characterized by the dysfunction of the fronto-striatal network. Fear reversal learning involves flexibly readjusting behaviour when circumstances change which means adapting from one predictive stimulus to another, largely depending on a fronto-striatal and fronto-amygdala network. The process of fear learning and extinction are thought to be related to the pathophysiology of anxiety disorders including OCD. Our study used functional magnetic resonance imaging (fMRI) concurrent with Galvanic Skin Responses (GSRs) to compare a sample of 18 OCD patients to 18 age matched healthy controls on a fear reversal paradigm. The results showed a clear bimodal distribution in the OCD patient group only. Compared to controls, half of the patients exhibited normal conditioning (significantly higher GSRs to the CS+ than the CS-), while the other half of OCD patients showed the opposite effect (significantly higher GSRs to the CS- than the CS+). Interestingly, during reversal learning, while the GSRs of healthy controls reflect flexible learning with significant higher responses to the new CS+ (previous CS-) compared to the new CS- (previous CS+), our OCD patient group shows an absence of differentiation between the reversed CS+ and CS-. In line with our hypothesis, we find inflexibility in fear learning in OCD patients, however the the presence of a bimodal distriubtion in the OCD patient group during conditioning suggest differences in emotional learning within OCD patients. Following these results we will investigate possible interactions with anxiety as well as with fronto-striatal and fronto-amygdala activations, which are currently being analyzed.

Disclosures: A.M. Apergis-Schoute: None. **C.M. Gillan:** None. **J.A. Blay:** None. **E. Fernandez-Egea:** None. **N.A. Fineberg:** None. **T.W. Robbins:** None. **B.J. Sahakian:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

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Program#/Poster#: 283.20/III17

Topic: F.01. Human Cognition and Behavior

Support: Stanford Neuroventures grant

Title: A Bayesian approach to model-based reinforcement learning

Authors: *E. M. MILLER[1], S. PIANTADOSI[2], N. GOODMAN[1], S. M. MCCLURE[1]; [1]Stanford Univ., Stanford, CA; [2]Univ. of Rochester, Rochester, NY

Abstract: Ever since it was discovered that dopamine neurons encode reward prediction errors in their firing rates, interest has focused on understanding the mechanism by which this signal facilitates learning (Schultz et al., 1998). One particular open question concerns the distinction

between model-based and model-free learning in the brain (Daw et al., 2005). Whereas in model-free learning, the actor is limited to encoding stimulus-response relationships between environmental cues and rewards, model-based learning allows one to benefit from higher level knowledge regarding the structure of the environment. The current study investigates the neural correlates of model-based learning in a category learning task. Optimal performance on the task required participants to learn to categorize stimuli based on three binary perceptual dimensions (shape, color, pattern). To model this type of learning, we employed a grammar-based Bayesian model of category learning that infers category membership based on previous evidence and prior beliefs about the structure of the task. Using this model, we generated a prediction error signal (surprisal) for each trial, representing the extent to which the evidence received deviated from participants' expectations. We found that this surprisal signal scaled with activation in the dorsal caudate and dorsolateral prefrontal cortex. Additionally, we found that Kullback-Leibler divergence, a measure of the extent to which the model's estimates of rule likelihoods changed after the receipt of new evidence, predicted activation in the dorsolateral prefrontal cortex after the receipt of surprising feedback, as well as during the implementation of this new information on the subsequent trial. These findings indicate that hypothesis updating during model-based reinforcement learning involves communication between the dorsal striatum and lateral prefrontal cortex.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Program#/Poster#: 283.21/III18

Topic: F.01. Human Cognition and Behavior

Support: NIH 5R01NS078784-02

Title: Neural correlates of model-based and model-free reinforcement learning strategies

Authors: *B. B. DOLL[1,2], K. D. DUNCAN[2], D. A. SIMON[1], D. SHOHAMY[2], N. D. DAW[1]; [1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Psychology, Columbia Univ., New York, NY

Abstract: Incremental learning across species is well described by reinforcement learning (RL) algorithms. The bulk of such demonstrations correlate behavioral and biological signals with signatures of model-free RL algorithms. This class of models is retrospective in nature, choosing

options based solely on outcomes previously obtained by selecting them. More recently, interest has grown in correlates of model-based RL which, by prospectively planning over a world representation, can exhibit more cognitive flexibility than model-free RL, though with greater computational cost.
Using fMRI, we investigated the neural correlates of learning in a task that dissociates model-based from model-free RL. Participants repeatedly navigated to terminal task states from different starting states in search of monetary reward. The states in this sequential task were represented by different classes of stimuli that activate unique regions of visual cortex. This task feature permitted us to search for neural correlates of RL strategies by searching for brain activations of different states in the sequential task.
Across the population, choice behavior showed evidence of both model-based and model-free learning. Preliminary fMRI results permitted closer investigation of the mechanisms by which these classes of learning algorithms are implemented. For each subject, we identified ROIs that showed preferential responses to the stimulus categories used to represent the different task states in an independent functional localizer. We then assessed the activity in these ROIs during the reward learning task start states. We looked for prospective activation of states to be navigated to, as well as retrospective reactivation of previous states visited. We observed significant reactivation of states visited in the previous trial. This reactivation was observed when these previous visitations terminated in a monetary gain, but not when they terminated in a loss. These results speak to the particular series of states subjects consider when engaging in model-based valuation, and suggest that this process may rely on replaying previous experiences rather than directly iterating a model of future state transitions.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Support: NIA 1R01AG041653

Title: Cue-approach training influences choice behavior via modulation of reward-related circuitry

Authors: *T. SCHONBERG[1], A. BAKKOUR[2], A. M. HOVER[3], J. PEREZ[3], L. NAGAR[3], J. A. MUMFORD[3,4], R. A. POLDRACK[3,4,5];
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Abstract: Deliberate exertion of effortful self-control often fails when trying to make life style changes, highlighting the importance of targeting automatic processes for behavioral change. We present a novel cue-approach task designed to influence subsequent choices of snack food items. Twenty five participants (16 females, ages 18-29, mean 20.7 ± 3.3) completed the task inside the MRI scanner (3T Siemens Skyra).
Participants were asked to fast for 4 hours prior to the experiment and participated in an auction that allowed us to obtain an individual measure of willingness to pay (WTP). We split the items based on the WTP's and defined 8 items as higher valued (HV) and 8 items as lower valued (LV).
The go-signal task is functionally the opposite of the stop-signal task. Images of 60 junk food items were presented on the screen for 1 sec. Participants were instructed to press a button as fast as they could only when they heard a tone. Sixteen (8 HV and 8 LV) out of the 60 items were consistently associated with the tone. The items were presented 12 times each during training. In the probe phase, participants were presented with pairs of items from the same value category (32 choices per type) that had similar values in the initial auction, but only one of the items in each pair was associated with the signal in the training phase (e.g. GoHV vs. NoGoHV). Participants were asked to choose one item per trial for a chance to consume that item at the end of the session. To measure the success of training we compared the ratio of choices of the Go vs. NoGo items (within HV or LV pairs).
We found that items associated with the go-signal during training were chosen significantly more during probe: for HV items (60% of trials p=0.008, LV items in 57% of trials, p=0.04).
In the imaging analysis we tested the modulation of the Go trials during training by the number of times each item was later chosen at probe and compared this modulation between the first and last runs of training. Data analysis and preprocessing were conducted using FSL5.
We found a modulation in value-related regions (precuneus and ventro-and medial prefrontal cortex) for the Go response of the HV items but only for the last training run but not the first. Thus, a cue-approach signal for individual items influenced subsequent choice via modulation of activity of value-related brain regions during training. Development of novel real-world behavioral change paradigms could benefit from this cue-approach technique to target automatic behavioral processes to bolster results and minimize relapse.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DA027764

Title: Neural circuitry underlying extinction learning with appetitive and aversive conditioned stimuli

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Abstract: On a daily basis, appetitive and aversive events are experienced in proximity to neutral stimuli; subsequently, these stimuli acquire affective properties. This type of conditioning is known to depend on the integrity of various subcortical regions involved in affective learning, such as the striatum and the amygdala. More recently, studies across species have begun to examine the neural circuitry underlying changes in conditioned responses once conditioned stimuli (CS) are presented in the absence of appetitive or aversive outcomes. This process, known as extinction, involves new learning or adapting of previously formed associations, and is essential in cases where CS elicit excessive, maladaptive emotional responses (e.g., fear in PTSD). However, it is unclear whether the extinction of appetitive and aversive CS depends on similar or distinct neural circuitry. In this study, we used fMRI to investigate the behavioral and neural processes underlying extinction learning with both appetitive and aversive CS. The experiment was framed as a game, using gain and loss of desirable items as appetitive and aversive outcomes, respectively. The use of instructed reinforcement in the context of the game ensured that appetitive and aversive outcomes were equated to one another. Participants underwent two blocks of a partial reinforcement Pavlovian conditioning procedure wherein four neutral fractals (CS) were each reinforced with a specific outcome that varied with respect to valence (gain, loss) and magnitude (high, low). In the extinction training that followed, all CS were presented in the absence of reinforcement. Preliminary analysis (n=10) suggests that both appetitive and aversive CS gained affective properties over the course of acquisition. These results were coupled with valence-specific differences in BOLD signals in the striatum during the initial acquisition phase. Behavioral results from the extinction phase suggest extinction of the affective properties gained by both appetitive and aversive CS during acquisition. During this phase, a valence by magnitude interaction was observed in cingulate cortex and bilateral insula, highlighted by increased activation in response to the less favorable outcome within each valence (e.g. low gain, high loss). Additionally, medial prefrontal cortex exhibited sensitivity to

magnitude, but not valence, during extinction. Further analyses will probe potential differences in connectivity between appetitive and aversive extinction circuits as well as changes over time in brain regions involved in affective learning.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Support: Mathers Foundation

Swiss National Fund

Title: Single-cell responses in human OFC, amygdala and ACC during two different neuroeconomic tasks

Authors: *M. R. HILL[1], E. D. BOORMAN[2], C. KOCH[1], I. FRIED[3];
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Abstract: In the current study we simultaneously recorded the spiking activity of multiple individual neurons in three different areas in the awake human brain known to be involved in neuroeconomic decision making; the orbitofrontal cortex (OFC), the amygdala and the anterior cingulate cortex (ACC). We conducted these recordings in patients with pharmacologically intractable epilepsy who were routinely implanted with depth electrodes for clinical diagnostics. We recorded data whilst subjects performed a classic slot machine task with random winning and losing outcomes. This task allowed us to find general neural correlates of winning and losing devoid of choice or learning. In a second experiment subjects had to pick a card from one of two decks. One deck had a 70% chance of winning, the other deck had a 30% chance of winning. Through trial and error the subjects could optimize their winnings by finding out which was the higher winning deck. Subjects played this game in turn with two virtual players. By watching the choices and outcomes of these two additional players the subjects could also acquire information about which of the two decks was the winning one.
We recorded from 116 units in the OFC, 148 units in the amygdala and 201 units in the ACC. In the slot machine task we found 4 units in the OFC, 9 in the amygdala and 12 in the ACC that responded to winning or losing. In the card game 7 OFC units responded to winning or losing

whilst in both the amygdala and the ACC we found 21 such responsive units respectively. From these responsive units only 1 OFC unit showed a significantly different response to winning vs. losing, whilst in the amygdala we found 9 such differentiating units and in the ACC 11. Amongst the responsive units, we found individual cells that significantly encoded in their firing rate the amount that was either won or lost ($10 or $100), the reward prediction error of the outcome or the outcomes and amounts won or lost by the two other players. We are collecting additional units to discover regional specialization of different neuroeconomic parameters.

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Poster

283. **Human Learning: Reinforcement and Reward**

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Topic: F.01. Human Cognition and Behavior

Title: Memory recall for high value items correlates with individual differences in white matter pathways associated with reward processing and fronto-temporal communication

Authors: N. REGGENTE, M. S. COHEN, Z. ZHENG, N. G. DE SHETLER, A. D. CASTEL, B. J. KNOWLTON, *J. RISSMAN;
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Abstract: How people manipulate their mnemonic abilities in a strategic manner (prioritizing the recall of material that would more likely produce a reward) is a complex phenomenon that integrates executive control and memory processes. Models of selective, reward driven behavior elucidate the diverse and complex interactions between prefrontal cortex, temporal lobe, and the mesolimbic dopaminergic reward circuitry. This study aimed to investigate the contributions of and communications between these systems to the recall of high value words.
With the adaptation of a value-directed memory paradigm, fMRI results showed that participants whose recall performance exhibited the greatest sensitivity to item value preferentially recruited left ventrolateral prefrontal cortex (VLPFC) and left lateral temporal cortex during the encoding of high value items. While this effect may partially be driven by individual differences in the strategic use of deep semantic encoding for high value words, it is also possible that structural differences in the connectivity between VLPFC and temporal lobe regions is a contributing factor. The Left Uncinate Fasciculus, a white matter tract that links the anterior parts of the temporal lobe with the ventral surfaces of the frontal lobe and a key pathway for semantic control (Harvey et al., 2013), is a likely candidate for an information highway between our two

fMRI result regions. As a measure of inferable integrity and efficiency of the white matter along the Left Uncinate, the Fraction Anisotropy (FA) was a strong correlate of subject's recall for high value words. This shows that not only were both regions from our fMRI results preferentially recruited for the encoding of high value items, but also the structural integrity of the white matter between them was a predictor of individual differences in recall performance. Given the strong role that item point values plays in determining subsequent recall performance, it is also of interest to examine the relationship between white matter pathways that connect core components of the brain's mesolimbic dopaminergic reward circuitry such as the nucleus accumbens (NAcc) and ventral tegmental area (VTA). Speaking to the richness of this circuitry's connections, the number of fibers that projected from the Left Nacc to the VTA was also a predictor for subject's recall for high value words. This study provides novel insights into the brain-behavior correlations between white matter connectivity between recruited functional regions and subsequent performance. Additionally, it speaks to the structural abundance of reward circuitry and its modulation of behavior.

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Poster

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William H. Neukom Institute for Computational Science

Title: Resting-state functional connectivity of automatically segmented nucleus accumbens in a large sample

Authors: *J. F. HUCKINS[1], T. F. HEATHERTON[2], W. M. KELLEY[2];
[1]Psychology and Brain Sci., [2]Dartmouth Col., Hanover, NH

Abstract: Introduction: The nucleus accumbens(NAcc) is an integral part of the mesolimbic-dopamine system, a substrate that is integral to a wide-range of functions including reward based learning. The NAcc is irregularly shaped and previous studies have used spherical, atlas based or

hand-drawn regions. The current study employs automated subcortical segmentation of the NAcc across a large sample and compares those results provides similar results to previous results with other methods for ROI identification.
Methods: Resting-state (10 minutes) and high-resolution T1 anatomical scans were obtained from 554 individuals. Standard preprocessing was performed in subject space with Data Processing Assistant for Resting-State fMRI (DPARSF), Resting-State fMRI Data Analysis Toolkit and custom scripts for scrubbing (Power et al., 2012) with spline interpolation to replace offending frames. FSL's FIRST automatically segmented NAcc ROIs from the T1, which were resliced to EPI resolution and functional connectivity maps were created per subject. Functional connectivity maps were normalized and smoothed. Group connectivity maps were created using SPM8.
Results: Visual inspection of ROIs created by FSL's FIRST showed corrected segmentation of the NAcc. Positively correlated resting-state functional connectivity results from the NAcc are similar to results seen from hand-drawn regions of interest in smaller sample sizes, prominently including contralateral NAcc, dorsal striatum, orbitofrontal cortex, medial prefrontal cortex, dorsal anterior cingulate cortex and medial cingulate cortex. Negatively correlated resting-state functional connectivity results from the NAcc were of smaller effect sizes and showed less overlap with previously published literature, prominently including visual cortex, precuneus, pons and temporal pole.
Discussion: Previous investigation of NAcc resting-state connectivity has relied upon hand-drawn, spherical or atlas based regions of interest. Automated segmentation of the NAcc using FSL's FIRST provides refined maps on an individual basis that can be applied to resting-state functional connectivity in a reliable manner with less manual intervention or possibility of human error. In summary, this study provides a method to interrogate resting-state functional connectivity of the nucleus accumbens in a reliable and automated way.

Disclosures: J.F. Huckins: None. **T.F. Heatherton:** None. **W.M. Kelley:** None.

Poster

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NSF Career Development Award

Title: Episodic memory interferes with reward learning and decreases striatal prediction errors

Authors: *G. WIMMER[1,2], E. K. BRAUN[2], N. D. DAW[3], D. SHOHAMY[2];
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Abstract: Learning from experience is central to adaptive decision making. Research on memory systems has demonstrated distinct cognitive and neural systems for learning stimulus-reward associations and for encoding episodes. In even simple experiences, however, these two types of learning often co-occur and may interact. Currently, it is unknown whether and how learning of stimulus-reward associations is influenced by memory for learning-related events. Here we sought to address this by examining how incremental reinforcement learning and reward-guided choices are influenced by episodic memory formation for the experience. During the experiment, participants made choices between two options (colored squares), each associated with a drifting probability of reward, with the goal to earn as much money as possible. Incidental, trial-unique object pictures, which were unrelated to the reward learning task, were overlaid on each option. The next day, participants were given a surprise memory test for these pictures.
We found that choices were significantly influenced by recent reward experience. Participants also exhibited significant memory for the pictures that were presented during learning, although they were unrelated to the reward learning task. This memory formation interacted with how reward guided choices: both across and within-participants, successful memory formation was associated with a decreased influence of recent reward experience on choice. Neurally, the canonical reward prediction error signal in the ventral striatum was decreased by successful memory formation and this decrease was preceded by enhanced functional connectivity between the hippocampus and striatum. These results demonstrate a mechanism by which reward-guided choices can be influenced by multiple memory systems. Further, they provide insight into the interactions between neural systems for reward learning and episodic memory.

Disclosures: G. Wimmer: None. **E.K. Braun:** None. **N.D. Daw:** None. **D. Shohamy:** None.

Poster

283. **Human Learning: Reinforcement and Reward**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 283.28/III25

Topic: F.01. Human Cognition and Behavior

Title: Why do we structure our knowledge? Two levels of rule generalization in reinforcement learning

Authors: *A. G. COLLINS, M. J. FRANK;
Brown University, CLPS, Providence, RI

Abstract: Even without supervision or incentives, people are able to acquire the complex rules that govern their environment. When learning stimulus-action mappings through reinforcement, they can structure their policy into abstract rules, and our prior behavioral and modeling work suggests that they seem have a naturally strong prior to do so, even when this does not afford any immediate advantage. Here we further investigate how and why individuals build such rules, outside of immediate usefulness, using our structured reinforcement learning model to derive and test behavioral predictions.
In a new reinforcement learning paradigm, subjects learned to select correct actions in response to stimuli presented in three different contexts. There were two stimulus-action rules, where one of them was valid in two contexts and the other only in a third context (but where each rule was equally frequent across trials). To test acquisition of structure, two subsequent phases introduced (i) new stimuli in old contexts, and then (ii) new contexts with old stimuli.
Consistent with model predictions, subjects transferred their self-constructed rule structure to new situations, at different processing levels. First, they were able to abstract away sensory context in favor of gathering rule-specific, rather than context-specific, knowledge, thus learning faster by clustering contexts cueing the same rule. Second, they learned faster in new contexts by generalizing known rules across stimuli. They thus transferred knowledge of self-built structure in two distinct ways. For a new stimulus, identification of the correct action in one context carried over to all contexts known to be associated with the same rule. Conversely, while in a new context, identification of the valid rule allowed transfer of a known rule to all stimuli in that new context. Moreover, when faced with a new context, subjects were more likely to reapply rules that were valid across multiple contexts than those that applied to only one, controlling for rule frequency. These results confirm our model's predictions, and show that the seemingly suboptimal strategy of building complex structure affords long term advantages given the opportunity to generalize across contexts in various sorts of new situations.

Disclosures: A.G. Collins: None. **M.J. Frank:** None.

Poster

283. Human Learning: Reinforcement and Reward

Location: Halls B-H

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant K99NS067241-01

NIH Grant MH55687

Dana Foundation

Title: Slow and fast-spiking neurons in the human substantia nigra are functionally distinct during reinforcement learning

Authors: *A. G. RAMAYYA[1], K. ZAGHLOUL[3], C. T. WEIDEMANN[4], B. LEGA[2], M. KAHANA[2], G. BALTUCH[2];
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Abstract: Animal studies of reinforcement learning (RL) have demonstrated that inhibitory interactions between slow-spiking, dopaminergic neurons and fast-spiking, GABA-ergic neurons in the midbrain are important during outcome evaluation. However, because animal studies typically study RL from primary rewards (e.g., juice), it is challenging to generalize these findings to human behavior, which is often motivated by secondary and tertiary rewards (i.e, higher-order rational, emotional and social goals). Here, recording from the substantia nigra (SN) of 41 Parkinson's patients performing a probability learning task with abstract feedback, we show that slow and fast-spiking neurons were anti-correlated in their temporal dynamics during the post-feedback interval. This functional distinction was absent when participants did not perform well during the task, suggesting that it may be related to RL. Our findings suggest that inhibitory interactions between dopaminergic and GABA-ergic neurons in the human midbrain may be important even when learning from higher-order, abstract outcomes.

Disclosures: A.G. Ramayya: None. **K. Zaghloul:** None. **C.T. Weidemann:** None. **B. Lega:** None. **M. Kahana:** None. **G. Baltuch:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.01/III27

Topic: F.01. Human Cognition and Behavior

Title: Intracranial spectral activity comparison between recognition and recall

Authors: *M. B. MERKOW[1], J. F. BURKE[2], M. J. KAHANA[3];
[1]Neurosurg., [2]Grad. Neurosciences Group, [3]Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: This study contributes to the longstanding debate of whether recall and recognition represent unique or overlapping memory processes. We sought to determine if the neural activity at encoding differentially predicts the success of retrieval between recall and recognition. Seven patients who underwent intracranial electrographic recordings for treatment of medically refractory epilepsy participated in a delayed free recall task. Following free recall, we tested subjects' ability to recognize the same words as the ones they had seen in the free recall task. This paradigm allowed us to compare spectral activity for words that were both recognized and recalled compared to those that were recognized but not recalled. Given the documented importance of the hippocampus for verbal memory function, we collected recordings from this anatomic region and surrounding structures. In the hippocampus, we found that low frequency activity decreased in recognized and recalled words relative to words that were recognized but not previously recalled. These results suggest that distinct neural activity in the medial temporal lobe occurs during successful encoding in the compared retrieval tasks.

Disclosures: M.B. Merkow: None. **J.F. Burke:** None. **M.J. Kahana:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.02/III28

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant # 1125683

Title: The influence of selective attention at encoding on post-retrieval monitoring

Authors: J. ARNDT[1], *A. L. DUARTE[2];
[1]Psychology, Middlebury Col., Middlebury, VT; [2]Georgia Inst. Technol., ATLANTA, GA

Abstract: We conducted an experiment examining how selective attention during encoding influenced the execution of post-retrieval monitoring processes. During encoding, pictures of objects were shown along with a scene source (a city scene, an outdoors scene, or an indoors scene) and a color source (red, green, or brown). Participants were asked to attend to one of the two sources on each encoding trial by deciding if the object was appropriate for the scene or

color that accompanied it. At retrieval, participants made old-new recognition judgments for each object, as well as source judgments for the scene and color that accompanied each object. Behavioral performance indicated that attention enhanced source memory, such that source memory performance was greater for attended than unattended sources. Event-related potentials (ERPs) were formed during retrieval for trials where participants correctly remembered an object was presented (hits) and 1) accurately remembered both sources (2 source correct), 2) accurately remembered the source to which they selectively attended during encoding only (1 source correct), and 3) remembered neither source presented during encoding (item only). Results demonstrated that a neural correlate of recollection the "parietal-old new effect" was modulated by the amount of source details retrieved with 2 source correct trials producing larger old-new effects than 1 source correct trials. Importantly, source retrieval accuracy modulated late right-frontal old-new effects often linked to post-retrieval monitoring, such that item only trials produced larger effects than 1 source correct and 2 source correct trials. These results suggest that when selective attention that is directed to an association between an object and a source fails to produce accurate source retrieval, as occurs for item only trials, people engage post-retrieval monitoring processes in an effort to recover the associations linking objects to their sources. In contrast, when attention during encoding successfully forms an association between an object and the sources encountered with it, as occurs for 2 source correct and 1 source correct trials, there is less of a need to engage post-retrieval monitoring processes compared to item only trials.

Disclosures: J. Arndt: None. **A.L. Duarte:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.03/III29

Topic: F.01. Human Cognition and Behavior

Title: Physiological relevance of estrogen effects on memory across the menstrual cycle of young women

Authors: A. POMPILI, M. D'AMICO, B. ARNONE, P. FEDERICO, *A. GASBARRI; Applied Clin. and Biotechnologic Sci., Univ. of L'Aquila, Dept. of Applied Clin. and Biotechnologic Scien, 67100 L'Aquila, Italy

Abstract: It is well known that brain encodes memories according to their emotional content and that emotional arousal influences the consolidation of long-term memory.

The aim of the present study was to verify the effects of estrogens on memory during the processing of affective pictures in young women, since the natural fluctuation of female hormone levels affect both physiological and psychological processes. Images from the International Affective System, based on valence (pleasant, unpleasant and neutral), were viewed passively by two groups of young women, one in a periovulatory phase (PO), characterized by high level of estrogens and low level of progesterone, and the other in early follicular phase (EF), characterized by low levels both of estrogens and progesterone. The electrophysiological responses to images were measured, data of visual evoked potentials were collected, and P300 peak was considered. One week after the presentation of emotional stimuli, long-term memory were tested by means of free recall.
As we expected, overall arousal images were better remembered compared to neutral pictures. However, the comparison between groups showed an interesting difference related to positive and negative stimuli: the PO women showed a significant better memory for positive images, while EF women showed a significant better memory for negative images. This could be explained as an effect of the levels of circulating estrogen, high in PO, and low in EF: memory is somehow modulated according to the phases of menstrual cycle. Moreover, results showed that women in the PO had better memory performance than women in the EF, and remembered a number of pictures significantly higher. The subjects in the PO phase showed greater P300 amplitude in parietal and frontal areas compared to women in EF, confirming that larger amplitudes correspond to a better ability to recall; therefore, amplitude of the P300 can be a predictive value of a better long-term memory consolidation.

Disclosures: A. Pompili: None. **M. D'Amico:** None. **A. Gasbarri:** None. **P. Federico:** None. **B. Arnone:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.04/III30

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH55687

Title: Multivariate algorithm for trial-by-trial estimate of goodness of encoding using human intracranial EEG

Authors: *J. BURKE[1], R. HAQUE[2], M. J. KAHANA[3];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania, Phiadelphia, PA;
[3]Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: The hallmark of episodic memory is mental time travel, or the ability to retrieve and re-experience past events. Episodic memory consists of the encoding, storage and retrieval of perceptual information. Behavioral studies indicate that the overlap between perceptual information at the time of encoding and retrieval is critical in the process of episodic re-experiencing. However, it is unclear where in the brain such overlap occurs. Here, we used intracranial electroencephalography (iEEG) from 98 neurosurgical patients participating in a delayed free recall task to investigate the spatial and spectral features that activated during both encoding and retrieval. We found that high-frequency activity increased during encoding and retrieval in a left hemispheric network consisting of the left ventrolateral temporal cortex, prefrontal cortex, and posterior parietal cortex. In addition, we found that theta oscillations in the right temporal cortex were specific to item retrieval and were not reliably observed during encoding. These data suggest that a spatiotemporally distinct subset of the neural activity that occurs during episodic encoding is recapitulated at the time of episodic retrieval.

Disclosures: J. Burke: None. **M.J. Kahana:** None. **R. Haque:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

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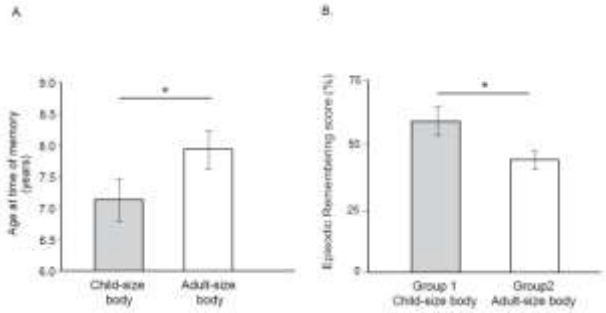
Wennergren Foundation

Title: Boosting recall of childhood memories with a child-size body illusion

Authors: *L. BERGOUIGNAN, B. VAN DER HOORT, H. H. EHRSSON;
Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: The default mode of encoding life-experiences is from the perspective of one's own body. However, because our body grows throughout development, the body-size context of the child during encoding is in discrepancy with the body-size context of the adult during recall. This could explain why adults have difficulties remembering their childhood memories. To test this hypothesis we introduce a novel experimental approach where healthy adult participants recalled childhood memories while they simultaneously experienced a multisensory illusion[1] of owning a child-size or adult-size artificial body in otherwise equivalent conditions. First, employing a within-subject design (n = 26), we showed that participants recalled significantly older negative memories when they experienced the child-size body illusion (t = -2.199; p = 0.037; see Figure, panel A). Next, constraining the age of the memories and using a between-subjects design (n =40), the child-size body illusion group showed a significantly increased episodic recall (t = 2.26; p = 0.029; see Figure, panel B). This study demonstrates that a child-size body illusion improves access to episodic childhood memory in adults. This suggests that the body-size context discrepancy between encoding and recall, which can be reduced by the child-size body illusion, contributes to the so-called childhood-amnesia phenomenon[2]. 1. Van der Hoort, B., Guterstam, A. & Ehrsson, H. H. Being Barbie: the size of one's own body determines the perceived size of the world. *Plos One* **6,** e20195 (2011). 2. Wetzler, S. & Sweeney, J. Childhood amnesia: a conceptualization in cognitive-psychological terms. *J. Am. Psychoanal. Assoc.* **34,** 663-685 (1986).



Disclosures: L. Bergouignan: None. **B. Van der Hoort:** None. **H.H. Ehrsson:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

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Program#/Poster#: 284.06/III32

Topic: F.01. Human Cognition and Behavior

Support: NIMH grant R01 MH069456

Title: Alternating study and retrieval practice leads to neural and behavioral differentiation of competing memory representations

Authors: *J. C. HULBERT, K. A. NORMAN;
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Abstract: Practicing retrieval of an item from memory can lead to impaired recall of related but unpracticed items (retrieval-induced forgetting; Anderson, Bjork, & Bjork, 1994). Retrieval-induced forgetting is commonly thought to arise as the result of inhibition, incited by the need to resolve competition between memories during selective retrieval. However, subsequent investigation has shown that the weakened competitors benefit disproportionately when retrieval practice is interleaved with opportunities to restudy the unpracticed materials. In some cases, this benefit can even lead to a reversed retrieval-induced forgetting effect (i.e., better recall of unpracticed competitors, relative to control items; Storm, Bjork, & Bjork, 2008). To explain this benefit, we hypothesized that the interleaving of competitive retrieval practice and restudy opportunities leads to differentiation of the neural representations of competing memories, thereby making memories of the items from the practiced category less confusable with one another and, through this, improving subsequent recall of both practiced and unpracticed items from this category. To evaluate this hypothesis, we measured the effects of interleaved rounds of retrieval practice and restudy using an fMRI sequence optimized to recover BOLD signal from the memory-related regions of the medial temporal lobe. Representational similarity analysis (Kriegeskorte, Mur, & Bandettini, 2008) was employed to measure the degree of neural overlap between competing memories. Using a combination of searchlight and region-of-interest analyses, we found that interleaved cycles of retrieval practice and restudy led to a decrease in neural overlap between competing memories (i.e., differentiation) in the hippocampus. Furthermore, this decrease in representational similarity predicted behavioral performance on the final test: participants showing greater levels of neural differentiation as a result of interleaved retrieval practice and restudy also showed the greatest behavioral benefit of this procedure on the recall of unpracticed competitors.

Disclosures: J.C. Hulbert: None. **K.A. Norman:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01-MH094480

Title: Time perception and contextual drift with a naturalistic stimulus

Authors: *O. LOSITSKY, D. TOKER, J. CHEN, C. J. HONEY, J. L. POPPENK, U. HASSON, K. A. NORMAN;
Princeton Univ., Princeton, NJ

Abstract: The question of how our experiences influence our perception of time has occupied experimental psychology for decades. In particular, what gives rise to our sense of duration on the order of minutes? Sahakyan and Smith (2013, in press) recently provided evidence that paradigms that induce changes in internal context can lead to greater retrospective duration estimates. In the present study, we further investigated how the evolution of mental context influences retrospective time estimates for episodes occurring on the order of minutes. As a proxy for mental context change, we used fMRI to track how distributed patterns of activity in the brain evolve gradually over time. Subjects were scanned while they listened to a 25-minute science fiction radio drama. Outside the scanner, subjects listened to pairs of audio clips from the story and were asked to estimate how much time had elapsed during the original story presentation between the first and second clips. Unbeknownst to participants, the actual time between clips varied between only two values: two and six minutes. As a result, the difference in time estimates within a condition could only arise from subjective factors. Subjects were reliably above chance at estimating the temporal distance between events, assigning longer durations to the six-minute intervals than to the two-minute ones. In addition, time estimates within a condition (two or six minutes) were consistent across subjects; that is, subjects tended to overestimate and underestimate the same intervals, despite them all being the same length. For our fMRI analysis, we approximated the "contextual similarity" between two clips as the correlation between brain patterns corresponding to those clips when they were originally played in the scanner. For each predefined region of interest, we correlated the similarity between brain patterns in that region with the subject's temporal proximity judgments. Preliminary results from these analyses show that greater pattern change was positively correlated with duration estimates in a right-lateralized set of brain regions, including the hippocampus, parahippocampal gyrus, basal ganglia and prefrontal cortex.

Disclosures: O. Lositsky: None. **D. Toker:** None. **J. Chen:** None. **C.J. Honey:** None. **J.L. Poppenk:** None. **U. Hasson:** None. **K.A. Norman:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant NS21135

Nielsen Corporation

NSF GRFP

Title: EEG and behavioral evidence for auditory directed forgetting in older adults

Authors: *M. E. CANO[1], R. T. KNIGHT[2,1];
[1]Helen Wills Neurosci. Inst., [2]Psychology, UC Berkeley, Berkeley, CA

Abstract: The neural basis of directed forgetting has received increased attention in the memory literature, with a focus on prefrontal cortex as the source of memory inhibition. The purposes of this study are twofold: 1) While the majority of directed forgetting studies have used visual stimuli, we seek to determine whether intentional forgetting extends to other sensory modalities 2) We aim to examine the behavioral and neuroelectric effects of auditory directed forgetting in older adults. To address these issues, we recorded electroencephalography (EEG) during a modified auditory version of Anderson and Green's Think/No-Think paradigm with healthy older adult participants. Participants were instructed to first learn auditorily presented word pairs. Successful initial learning was followed by a recall test to confirm sufficient learning. In the next phase of the experiment, participants were again presented with the first word of each pair, accompanied by a visual instruction cue consisting of a red or green box, which indicated either to silently retrieve the second word of the pair (green box) or to inhibit recollection of the second word of the pair (red box). Subsequent memory was later tested with same-probe and independent-probe memory tests to determine the effect of instruction and active control of memory on later memory. Here, we examine task- related EEG activity during covert intentional memory retrieval and inhibition. Behaviorally, older adults showed a directed forgetting effect similar to that seen in previous studies using young adults, such that No-Think words were forgotten at a higher rate than baseline and Think words ($p<.05$ and $p<.01$, respectively), demonstrating successful active memory suppression for No-Think items. ERPs were examined during two periods of the Think/No-Think phase of the experiment and were time-locked to instruction cue onset and word onset. Cue-related activity produced a fronto-central ERP negativity for No-Think compared to Think trials (200-300 ms, $p<.05$), which may reflect the process of general preparatory inhibition. Word-related activity produced a sustained and widespread ERP negativity for No-Think compared to Think trials ($p<.05$), potentially reflecting the active inhibition of the particular item in memory. The behavioral and ERP data suggest that auditory directed forgetting elicits similar effects to those seen in the visual directed forgetting literature, and that those effects can also be elicited in older adults.

Disclosures: M.E. Cano: None. **R.T. Knight:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

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Topic: F.01. Human Cognition and Behavior

Support: DFG grant AX82/2-1

Title: Reinstatement of content-specific intracranial EEG activity during retrieval in a spatial navigation task

Authors: *H. ZHANG[1], J. FELL[1], B. STARESINA[1,2], N. AXMACHER[1,3];
[1]Dept. of Epileptology, Univ. of Bonn, Bonn, Germany; [2]Med. Res. Council Cognition and Brain Sci. Unit, Cambridge, United Kingdom; [3]German Ctr. for Neurodegenerative Dis., Bonn, Germany

Abstract: Retrieval of episodic memories depends on the reinstatement of encoding-related activity. Representational similarity analysis (RSA) is a straightforward method to analyze the similarity between neural activity patterns based on correlations. Indeed, previous RSA studies have shown that stimulus-specific neural activity patterns re-occur during subsequent retrieval of the same information, however, the exact time-frequency signature of this process still remains to be investigated. Here, we explored the reinstatement of content-specific activity patterns in ten pre-surgical epilepsy patients. Depending on their clinical background, patients were implanted either with medial temporal depth electrodes or subdural grid and strip electrodes or both (average number of electrode contacts: 19.5; range: 9~32). In a first-person virtual navigation experiment, patients first encoded and then retrieved their route through a virtual furnished house. We extracted time-frequency activity patterns across all electrodes in each patient. Then, for each time-frequency bin, we calculated correlations across electrodes between encoding and retrieval of the same room in a house as compared to encoding of one room and retrieval of a different room. We found that encoding-related activity was reinstated in a content-specific manner during retrieval. Specifically, reinstatement occurred mainly in the high (gamma) frequency range between 30-80 Hz, and only before the decision point in each virtual room. This suggests that encoding-related activity is only recalled when subjects need to remember their virtual route. Taken together, these data are the first to delineate the specific time-frequency pattern of content-specific memory reinstatement.

Disclosures: H. Zhang: None. **J. Fell:** None. **B. Staresina:** None. **N. Axmacher:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

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Program#/Poster#: 284.10/III36

Topic: F.01. Human Cognition and Behavior

Support: R01 MH055687

R01 MH061975

Title: Neural correlates of memory encoding as a function of practice

Authors: *N. M. LONG[1], M. J. KAHANA[2];
[2]Psychology, [1]Univ. of Pennsylvania, Philadelphia, PA

Abstract: The neural correlates of successful memory formation, although well characterized, are typically the result of single session data. In contrast, memory formation outside of the confines of the laboratory is the result of a lifetime of practice, leaving open the question of whether the same mechanisms that support initial one-shot learning support memory formation across multiple study episodes. To address this issue, we collected scalp EEG as subjects studied and freely recalled lists of words across 7 sessions on separate days. Recall accuracy increased across sessions, as did subjects' tendency to exhibit a contiguity effect. Analysis of the spectral correlates during encoding showed that the contrast of successfully encoded to forgotten items was characterized by significant power decreases across low frequencies, an effect which increased significantly across sessions. Temporal clustering, the tendency to consecutively recall study neighbors, was characterized by an increase in gamma power across sessions. Taken together, these results suggest that mechanisms underlying successful memory encoding and subsequent temporal clustering become more pronounced with practice.

Disclosures: N.M. Long: None. **M.J. Kahana:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

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Program#/Poster#: 284.11/III37

Topic: F.01. Human Cognition and Behavior

Support: NRSA F32 EY021999

NIH R01 EY021755

Title: Interacting signals of memory during competitive encoding

Authors: *J. HUTCHINSON, N. B. TURK-BROWNE;
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Abstract: Although memory encoding and retrieval are often studied as separate entities, old and new items are often encountered at the same time. The deployment of attention in such competitive situations is varied, including being biased to repeated information in some settings and to novel information in others, depending on the memory system(s) involved. Using fMRI of the human brain, the current study sought to isolate the contributions of novel and repeated information to attention and memory systems during competitive processing. Specifically, competition was induced by presenting participants with a face image at central fixation, which was surrounded in the periphery by a scene image. The face image had either been encountered recently or was novel, and participants were given a subsequent memory test for the competing scene stimuli. This design allowed us to examine: (1) behavioral memory for novel scenes as a function of whether they were presented in competition with novel or repeated faces, (2) neural evidence of priming for the novel vs. repeated faces, as reflected in attenuated evoked responses in face-selective visual cortex, (3) neural evidence of encoding for the novel scenes, as reflected in the activity of scene-selective visual cortex, and (4) the interaction of these measures both within visual cortex and more broadly in attention networks. Preliminary results suggest that neural activity in ventral temporal regions and beyond is differentially related to memory outcome for the scenes, depending on whether there is competition from repeated or novel faces. Such findings indicate that the encoding of novel information can be influenced by signals from multiple memory systems about the mnemonic status of other currently presented items.

Disclosures: J. Hutchinson: None. **N.B. Turk-Browne:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

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Title: Brain structural differences associated with mnemonic control

Authors: *G. LERMA-USABIAGA[1], L. GARCIA-PENTON[1], M. CARREIRAS[1,2,3], P. PAZ-ALONSO[1];
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Abstract: A component of goaldirected behavior is the ability to focus on memories that are relevant to our current goals. The Think/No-Think (TNT) paradigm is a useful tool to examine mnemonic control as it is designed to study the effects of intentional attempts to bring or to not bring to mind information from long-term memory. Behavioral research suggests that efforts to suppress memory retrieval are associated with a reduction in memory for these associations in a subsequent test. Recent neuroimaging evidence has showed that compared to individuals who were not able to suppress memory retrieval, participants who did so exhibit tighter coupling between key nodes in a dorsolateral prefrontal cortex-cingulate-parietal-hippocampal network which cortical inputs may be mediated by the cingulum bundle (Paz-Alonso et al., 2013). Also, a recent study has shown that suppression of memory retrieval is associated with overall reduction of hippocampal volume (Depue et al., 2011). Based on prior evidence suggesting the involvement of the cingulum bundle in mnemonic control and longaxis specialization within the hippocampus (Poppenk et al., 2013), the present study was aimed at investigating if volume differences in anterior versus posterior hippocampus and the integrity of the main white-matter pathways connecting the hippocampus and the prefrontal cortex are associated with memory suppression. A total of 27 healthy young adults participated in the study. Our procedure involved three phases. First, participants learned a series of word-pairs (Vacation-Palm). Second, they were presented with the first word of the pair (Vacation), and asked to either remember (Think condition) or suppress (No-Think condition) the second word (Palm). Finally, participants were encouraged to recall all of the studied item-pairs. fMRI data was acquired between the learning and the testing phases. As expected, behaviorally, participants exhibited a lower percent recall for No-Think items compared to Baseline items (items that were learned but were not included in Think/No-Think phase. Our neuroimaging results revealed that reduced anterior, but not posterior, hippocampal volume was associated with memory suppression. These results support prior evidence of a longaxis specialization in the hippocampus and qualify prior findings (Depue et al., 2011). Furthermore, probabilistic tractography analysis revealed that white-matter integrity of the bilateral cingulum bundle, but not of the uncinate fasiculus, was associated with memory

suppression. These results constitute the strongest and most detailed evidence so far for the relation between brain structure and mnemonic control.

Disclosures: G. Lerma-Usabiaga: None. **L. Garcia-Penton:** None. **M. Carreiras:** None. **P. Paz-Alonso:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.13/III39

Topic: F.01. Human Cognition and Behavior

Title: fMRI examination of motivated learning and memory of scenes in adults and adolescents

Authors: *A. MATTFELD[1], M. SHERMOHAMMED[2], J. D. E. GABRIELI[2];
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Abstract: We used fMRI to investigate the neurobiology of reward-motivated learning and memory in adults and adolescents during encoding and retrieval. Systems neuroscience has identified a network of regions including midbrain dopaminergic neurons, ventral striatum and orbito- and medio-frontal cortices that are important for reward processing. Similarly, a network of regions has been identified as important for the acquisition and subsequent expression of declarative memory including the medial temporal lobe (MTL), parietal, and prefrontal cortices. The interaction between these two networks and their changes across development have been unexplored. Here, we used the monetary incentive encoding (MIE) paradigm to examine motivated learning and memory in adults and adolescents. During the MIE task, participants were required to learn scenes for a subsequent memory test, with a cue preceding each scene indicating whether a large reward ($5) or a small reward (10¢) would be received for later remembering that scene,
Behaviorally both adults and adolescents showed greater memory, as measured by d-prime, for scenes paired with large relative to small rewards. Adults during the reward cue period showed activation across the reward network including the medial prefrontal cortex, dopaminergic midbrain, and bilateral caudate. There was also activation in bilateral hippocampus and right parahippocampal cortex for successfully remembered compared to forgotten scenes during the target scene period. These findings suggest that during encoding, motivated learning and memory recruit distinct reward and memory networks. Evaluation of their interactions and how these interactions change across development will provide useful insight into the motivated learning and memory circuitry.

Disclosures: A. Mattfeld: None. **M. Shermohammed:** None. **J.D.E. Gabrieli:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.14/III40

Topic: F.01. Human Cognition and Behavior

Support: AX82/2-1

Title: The oscillatory signature of voluntary forgetting: Intracranial EEG data from prefrontal cortex and hippocampus

Authors: *C. OEHRN[1], J. FELL[1], C. BAUMANN[1], S. HANSLMAYR[2], M. LESZCZYNSKI[1], A. JAHANBEKAM[1], A. T. DO LAM[1], C. E. ELGER[1], N. AXMACHER[1];
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Abstract: Forgetting is commonly regarded as a limiting rather than as a valuable feature of our memory system. However, forgetting can help us to eliminate unwanted, irrelevant or emotionally straining information,
and thus enables a flexible and goal-directed use of memory resources. A powerful way to keep our memory clean is to prevent the formation of unwanted memories in the first stage, during encoding. This process can be driven intentionally, as shown in the item-method directed forgetting paradigm, where participants are cued to forget individual items briefly after presentation. We investigated the time-frequency pattern underlying this form of voluntary forgetting by recording intracranial EEG from the dorsolateral prefrontal cortex (DLPFC) and the hippocampus in 22 epilepsy patients undergoing pre-surgical evaluation. We found that the "forget" instruction triggered an increase in gamma-band activity (30-150 Hz) in the DLPFC and in the hippocampus. Furthermore, we observed that voluntary forgetting was associated with increased activity in the beta (13-18 Hz) frequency range, specifically in the anterior hippocampus. These results are the first to draw a detailed picture on the oscillatory signature underlying voluntary forgetting and suggest that forgetting is reflected by an increase in prefrontal gamma band activity and increased gamma and beta band activity in the hippocampus.

Disclosures: C. Oehrn: None. **J. Fell:** None. **C. Baumann:** None. **S. Hanslmayr:** None. **M. Leszczynski:** None. **A. Jahanbekam:** None. **A.T. Do Lam:** None. **C.E. Elger:** None. **N. Axmacher:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.15/III41

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant IIS-1009542

Title: Recall order is predicted by category-specific neural activity of preceding items at study

Authors: *S. C. Y. CHAN[1], M. C. APPLEGATE[1], J. R. MANNING[1], N. W. MORTON[2], S. M. POLYN[2], K. A. NORMAN[1];
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Abstract: Context-based models of episodic memory posit that memories of items are linked to a "mental context" representation that drifts over time; at test, we can retrieve recently-studied items by cuing with the current state of mental context. These models have successfully explained a wide range of memory effects (e.g., temporal clustering in free recall), but there is still considerable debate over the factors that shape mental context. Early context models (e.g., Estes, 1955) posited that mental context drifted randomly over time, whereas modern implementations (e.g., Howard & Kahana, 2002) posit that mental context reflects a running average of recently-experienced thoughts. A key prediction arising from the latter set of models is that, if two items are preceded by similar thoughts, they will be linked to similar mental contexts, which in turn will bias participants to recall the items sequentially at test. To test this prediction, we scanned participants in an fMRI scanner while they studied, one item at a time, lists of the form AACBBCAACBBCAACBBC, where A, B, and C denote different categories. For each list, participants were asked to recall as many C-items as possible, in any order. We used an fMRI pattern classifier to track category-specific neural activity, and examined pairs of C-items that were preceded by the same category during study (e.g. A). For a given pair, if the classifier showed an elevated level of the preceding category when the two C-items were studied, the participants showed an elevated probability of recalling the two items sequentially, as predicted.

Disclosures: S.C.Y. Chan: None. **M.C. Applegate:** None. **J.R. Manning:** None. **N.W. Morton:** None. **S.M. Polyn:** None. **K.A. Norman:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.16/III42

Topic: F.01. Human Cognition and Behavior

Support: AX82/2

Title: Periodic interplay between inhibition and maintenance in the human hippocampus during working memory task

Authors: *M. LESZCZYNSKI[1], J. FELL[1], C. OEHRN[1], A. DO LAM[1], C. E. ELGER[1], N. AXMACHER[1,2];
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Abstract: Recent evidence suggests that the hippocampus does not only contribute to long-term memory encoding and retrieval, but also supports working memory (WM) processes. Here, we used intracranial EEG recordings in epilepsy patients to describe properties of the hippocampal contribution to WM. We observed a load-dependent increase of activity in the gamma frequency range (30-150Hz), possibly indicating enhanced recruitment. Furthermore, we found that activity in the alpha/beta frequency range (8-20Hz) decreased with load, putatively reflecting release from inhibition. Interestingly, the latter effect was not sustained across the entire maintenance period, but occurred during four clusters that were interrupted by periods of similar length during which no alpha/beta load effect was observed, but which, in contrast, showed a load-independent increase of alpha/beta power as compared to baseline.
We further tested several mechanistic predictions derived from the multiplexing buffer model of WM (Lisman & Idiart, 1995; Lisman & Jensen, 2013). First, we found that the amplitude of high-frequency activity depends on the phase of simultaneous low-frequency oscillations (cross-frequency coupling, CFC), as previously observed during maintenance of faces. We observed a pronounced phase-to-power coupling between the phase of theta oscillations (4-6Hz) and the power of alpha/beta oscillations (8-20Hz), as well as between theta phases and gamma power (30-50Hz) and between alpha/beta phase and gamma power. Across all clusters, the average CFC ratio was at around 7 and thus significantly higher than the CFC ratio during maintenance of faces, which was at 4. Most importantly, we found that CFC was not constant across the maintenance period but fluctuated between states of high and low CFC strength. This fluctuating pattern was complementary to the previous alpha/beta load-dependent effect: Periods showing load-dependent release from inhibition displayed lower CFC values, whereas periods with load-independent alpha/beta enhancement were characterized by higher CFC values. This difference cannot be explained by a change in overall (load-independent) power, which was identical for both intervals. These results suggest a refined model for WM maintenance depending on a

periodic interplay between periods of pronounced cross-frequency coupling and periods of load-dependent release from inhibition.

Disclosures: M. Leszczynski: None. **J. Fell:** None. **C. Oehrn:** None. **A. Do Lam:** None. **C.E. Elger:** None. **N. Axmacher:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.17/III43

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant BCS0745880

Title: Memory for shape reactivates the lateral occipital complex

Authors: *J. M. KARANIAN, S. D. SLOTNICK;
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Abstract: Memory is thought to be a constructive process in which the cortical regions associated with processing the features of the preceding event are reactivated during retrieval. Although there is evidence for non-detailed cortical reactivation during retrieval (e.g., memory for visual or auditory information reactivates the visual or auditory processing regions, respectively), there is limited evidence that memory can reactivate cortical regions associated with processing detailed feature-specific information. Such evidence is critical to our understanding of the mechanisms of episodic retrieval. The present functional magnetic resonance imaging (fMRI) study assessed whether the lateral occipital complex (LOC), a region that preferentially processes object shape, is associated with the accurate recall of shape information. At encoding, participants were presented with colored abstract shapes that were either intact or scrambled. At retrieval, colored disks were presented and participants indicated whether the corresponding shape was previously “intact” or “scrambled”. A random-effect general linear model analysis was conducted. Activity associated with the recall of intact shapes was isolated by contrasting “intact”/intact > “scrambled”/intact, and activity associated with the recall of scrambled shapes was isolated by contrasting “scrambled”/scrambled > “intact”/scrambled (i.e., hits > misses). Preliminary analysis revealed that the recall of intact shapes, but not scrambled shapes, activated the LOC. Furthermore, LOC activity was significantly greater during the recall of intact than scrambled shapes. The present results suggest that the LOC is preferentially associated with retrieval of shape information. This feature-specific evidence supports the view that retrieval is constructive in nature.

Disclosures: J.M. Karanian: None. **S.D. Slotnick:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 284.18/III44

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant T32-AG020418

Title: Oscillating auditory stimulation during sleep promotes sleep spindles

Authors: *J. W. ANTONY[1], J. BAE[2], K. A. PALLER[1,2];
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Abstract: Two types of oscillations play particularly important roles in memory processing during sleep. Slow oscillations (>75 μV, 0.5-2.0 Hz) occur most prominently during slow-wave sleep (SWS). Spindles are bursts of oscillatory activity between 11-16 Hz lasting 0.5-2 seconds occurring during stage-2 and SWS. Spindles occur non-uniformly across the scalp, with slow spindles (11-13.5 Hz) more prominent at frontal regions and fast spindles (13.5-16 Hz) more prominent at centroparietal regions. Several methods have been used to show that facilitating slow waves can enhance memory, perhaps due to indirect effects on spindle activity. Here we attempted to manipulate spindles directly by presenting oscillating white-noise bursts at spindle frequencies during sleep. Two groups of 11 young, healthy subjects napped in the afternoon for up to 90 minutes with an auditory background of white noise. Following EEG signs of stage-2 or SWS, we presented 2-second bursts wherein the amplitude of white noise oscillated sinusoidally between 20-100% of the background amplitude at 15 Hz in one group and 12 Hz in the other (corresponding roughly to fast and slow spindles, respectively). Each burst was followed by 8 seconds of white noise at the background amplitude. Spindles were detected using an established automated algorithm. During 15-Hz stimulation, there was an increase in fast spindles relative to subsequent non-stimulation periods. Slow spindles did not differ between the stimulation and non-stimulation periods. During 12-Hz stimulation, there was only a trend for an increase in slow spindles. Between-group comparisons confirmed that spindle frequency varied reliably as a function of stimulation frequency. Further analyses compared spindles during stimulation to spindles following stimulation. No differences were found in EEG power spectral density, duration, or topography, suggesting that induced spindles were physiologically similar to spontaneous spindles. Prior findings have linked spindles with memory consolidation, general

intelligence, and sleep stability. This successful demonstration of a method to facilitate spindles selectively and non-invasively opens the door to new research into the physiological functions of spindles and to practical applications of spindle induction methods.

Disclosures: J.W. Antony: None. **J. Bae:** None. **K.A. Paller:** None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

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Topic: F.01. Human Cognition and Behavior

Support: NSF/SLC grant

Title: Evolution of hippocampal involvement in tactile-kinesthetic memory encoding and retrieval

Authors: *L. T. LIKOVA;
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Abstract: Introduction. Recently, we developed a memory-training paradigm based on non-visual drawing and found dramatic improvements in memory-guided spatiomotor performance in both congenitally blind and blindfolded (sighted) subjects (Likova 2012, 2013) with a one-week training protocol. To assess the evolution of hippocampal involvement, we ran a pre/post-training analysis of the memory-related activation. Methods. The tasks were: encoding through tactile exploration and memorization (EM) of the complex spatial images to be drawn, memory-retrieval through drawing guided solely by tactile memory (MD), and control scribbling (S), each of 20s duration, separated by 20s rest-intervals. FMRI (Siemens 3T scanner) was run before and after a week of drawing training in the congenitally blind, and also following a prolonged consolidation period. A fiber-optic motion-capture system recorded the drawing movements. Results and Conclusions. The hippocampal region, which was activated in encoding (EM) but strongly deactivated in memory retrieval (MD) before training, reversed its sign to become strongly activated in retrieval after training and consolidation, but non-responsive in encoding. Interestingly, this dramatic reversal was highly correlated with the changes of the response pattern in the primary visual area V1, which we have previously implicated as the (amodal) spatial-sketchpad for working memory. As expected, no response was observed in these regions for the control (S) condition either pre- or post-training. The progression of the hippocampal/V1 response reorganization was well coordinated with a differentiation into non-overlapping encoding and subsequent retrieval networks throughout the inferotemporal cortex (IT). The

observed training-driven evolution of this rapid functional reorganization carries important implications for theories of the role of the hippocampus, as well as of V1 and IT, in human learning and memory encoding and retrieval, with particular reference to cross-modal brain plasticity under tactile-kinesthetic guidance, and to potential strategies for blindness rehabilitation.

Disclosures: L.T. Likova: None.

Poster

284. **Human Long-Term Memory: Encoding-Retrieval Interactions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: IN220712

IN217311

Title: Genotypes of the rs2180619, located on the regulatory region of the CNR1, exhibit differential accuracy during encoding but not during retrieval in an episodic memory task

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Abstract: We have demonstrated that individual differences in cognitive performance, such as attention, procedural learning and working memory are associated to genetic polymorphisms of the CNR1 gene, which codes for Cannabinoid receptor 1 (CB1R). However, association of polymorphisms of CNR1 and encoding and retrieval performance of episodic memory remains unknown, and seems plausible because CB1R are highly distributed in hippocampus, which is involved in episodic memory. Studies in animals and human subjects have revealed a functional role of endocannabinoid system in episodic memory. Here, we studied the association of the genotypes of the rs2180619 of the CNR1, a polymorphism located in a regulatory region of the

CNR1, and the performance on an encoding-recognition episodic memory task. The alleles of the rs2180619 are A>G; the G allele has been associated to addiction and high levels of anxiety (when G allele interacts with the SS genotype of the 5-HTTLPR gene). One-hundred and sixty four participants were genotyped (AA, n=41; AG, n=90; GG, n=33), and solved an encoding and a recognition episodic-memory task. During encoding phase, subjects had to categorize 60 words between concrete and abstract meaning (50% of each category). Immediately after and surprisingly for subjects, a recognition task was administered; subjects had to discriminate between old (presented during encoding phase) and new words. Only during encoding phase, a differential performance was associated to genotypes of the rs2180619: GG subjects did not show difference in speed processing of concrete vs. abstract words, whereas AA and AG subjects responded faster to concrete than to abstract words, as previously has been reported. Despite this differential performance in speed during encoding, no differences among genotypes were found in performance during recognition. Our results suggest that performance of genotypes of the rs2180619 is more related to a semantic processing than to an episodic one.
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Poster

285. **Language II**

Location: Halls B-H

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Program#/Poster#: 285.01/JJJ1

Topic: F.01. Human Cognition and Behavior

Support: MRC Grant G0400341

Waterloo Foundation

Title: The effects of perceptual distortion, age and proficiency on functional activation for sentence comprehension

Authors: *S. KRISHNAN[1], R. LEECH[2], E. MERCURE[3], S. LLOYD-FOX[1], F. DICK[1]; [1]Birkbeck, Univ. of London, London, United Kingdom; [2]Imperial Col., London, United Kingdom; [3]UCL, London, United Kingdom

Abstract: In the school years, children become proficient language users. They acquire a larger vocabulary, and start to comprehend and use more complex syntactic constructions. During the same developmental period, perceptual, attentional and cognitive abilities are changing. These changes influence children's language comprehension ability. A gradual refinement of syntactic comprehension occurs in tandem with refinements of perceptual and attentional ability. In adverse listening conditions, this trajectory for sentence processing is extended even further (Leech et al., 2007). The functional neural bases of these protracted behavioural changes in language comprehension are not well understood. In adults, syntactic comprehension is associated with activation in a well defined set of regions. Nonetheless, this activation can vary with relation to sentence complexity & task demands. Only a handful of studies focus on developmental differences in syntactic comprehension (Yeatman et al., 2010; Nunez et al., 2011). These studies focus on neural effects related to syntactic complexity alone. However, despite children's everyday exposure to noisy & distracting environments (such as classrooms/ playgrounds), the effects of perceptual/attentional factors on neural activation for language remain largely unexplored.
We compared school-age children (7-13 year olds, N = 38) and adults (N = 18) to characterise developmental differences in the neural activation for sentence comprehension.In our fMRI task, participants had to identify the agent of a sentence. Sentence complexity as well as perceptual/attentional demands were modulated. Complexity was modulated by using simple (active/ subject clefts) & more complex sentences (passives/ object clefts). Perceptual/attentional demands were increased by introducing speech compression plus low-pass filters (Dick et al., 2001). To identify potential interactions, we explored the relationships between neural activation, age, & performance on a range of auditory-motor behavioural tasks. All conditions elicited activation in the superior temporal and inferior frontal regions in both groups. Perceptual distortion was associated with decreased activation in the superior temporal regions. Although overall patterns of activation were similar, group differences were observed when comparing children/adults. These indicate that the functional organisation of language comprehension in schoolchildren is still undergoing significant change. We also discuss the complex interplay between individual differences in auditory/ syntactic proficiency on children's neural activation for sentence processing.

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Poster

285. **Language II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.02/JJJ2

Topic: F.01. Human Cognition and Behavior

Support: Australian Research Council

Australian Postgraduate Award

Title: Recognition of novel words learnt with levodopa modulates striatal and temporal cortical activity

Authors: *A. RAWLINGS[1], K. MCMAHON[2], A. MACDONALD[1], E. FINCH[3], P. SILBURN[1], P. NATHAN[4], D. A. COPLAND[1];
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Abstract: Background: Levodopa, a dopamine precursor, has been shown to improve new word learning in healthy adults. However the neural and cognitive mechanisms underlying this effect remain unclear. This study aimed to investigate this effect using a double-blind, placebo-controlled drug study in young healthy adults. Participants learnt new names for familiar objects over a four-day period, followed by a functional magnetic resonance imaging (fMRI) task examining recognition of newly learnt words.
Methods: 33 (17 female) healthy young adults were randomly assigned to one of two drug arms. Participants received a tablet (Madopar 125mg or placebo) prior to daily training sessions in a novel explicit word learning paradigm over four consecutive days. Participants learnt new auditory names (legal nonwords) for 50 commonly-known familiar objects. After completion of the four training sessions, recall and recognition of the newly-learnt words was tested while event-related fMRI data was acquired with blood oxygen level-dependent (BOLD) contrast at 4 Tesla. All image processing and analysis was conducted in SPM8.
Results: Region-of-interest analysis (using $p < .05$ corrected) showed that while performing the new word recognition task, participants who had taken levodopa exhibited increased activity in the left striatum and left hippocampus as compared to participants who had taken a placebo. Additionally, participants in the levodopa group exhibited less activity in left superior and middle temporal gyri compared to participants in the placebo group.
Conclusions: The results suggest that levodopa may modify learning of new words through increased recruitment of the left striatum and hippocampus, two primary targets of dopaminergic signalling that are strongly implicated in learning and memory. The decreased temporal cortex activity observed for the levodopa group suggests more efficient retrieval of recently acquired word representations. This research contributes to our understanding of the role of dopamine in language learning and could assist with the development of novel pharmacological treatments for language disorders such as post-stroke aphasia.

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Poster

285. **Language II**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.03/JJJ3

Topic: F.01. Human Cognition and Behavior

Title: Involvement of left inferior frontal cortex in processing compound words for lexical decision

Authors: *W.-J. KUO;
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Abstract: During the course of learning to read, the readers need to develop a mechanism to regulate computation of various representations and the way to get access to their mental lexicon. Studies have shown that the
mechanism is often complicated by the trade-off between storage and computation for the representation and processing, as for the processing of compound words (Taft and Forster, 1976; Butterworth, 1983; Marslen-Wilson et al., 1994). In Chinese, over 80% of Chinese words are two-character compounds (Tsai et al., 2011). For content words, the meaning of a two-character compound can be either semantically transparent or opaque to its constituent characters. As recognized by research in this regard, semantic transparency is an important factor to affect dynamics of compounding process (Andrews, 1986; Zwitserlood, 1994; Libben, 1998; Libben et al., 2003; Fiorentino and Poeppel, 2007). In this study we specifically designed an fMRI experiment to explore neural bases of this lexical control mechanism for word compounding by using Chinese two-character compounds. The results indicated that processing the opaque words needs more time than the transparent words, and it interacts with their neighborhood size. For opaque words, the larger their neighborhood size the more time it takes for their processing. For the transparent words, the opposite response pattern was observed. Interestingly, the left inferior frontal cortex (BA 44, the Broca's area) was involved in this type of morphological computation by mirroring its activity pattern in the same way.

Disclosures: W. Kuo: None.

Poster

285. **Language II**

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Topic: F.01. Human Cognition and Behavior

Support: NSF Grant IIS-1208203

Dingwall Neurolinguistics Fellowship

Title: Cortical organization of semantic representations for natural speech revealed by fMRI

Authors: *A. G. HUTH[1], W. A. DE HEER[2], F. E. THEUNISSEN[2], T. L. GRIFFITHS[2], J. L. GALLANT[2];
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Abstract: The human brain is adept at extracting meaning, or semantic content, from natural speech. Low-level speech features such as phonemes are known to be processed in specialized areas of auditory and motor cortex. In contrast, previous research suggests that semantic processing is widely distributed across the brain, involving much of the temporal, parietal, and frontal lobes. However little is known about how different aspects of semantic information are mapped systematically across the cortical surface.

To address this issue we collected BOLD fMRI responses from five subjects while they listened to over two hours of natural speech (stories from "The Moth Radio Hour"). Each word in the stimulus was projected into a 1000-dimensional semantic feature space constructed using word co-occurrence statistics from a large corpus of text. We used this feature representation to build a separate L2-regularized linearized finite impulse response (FIR) regression model for each voxel in each subject's brain. These models predict the response of each voxel as a linear combination of the 1000 semantic features.

To find a compact, meaningful representation of these models we applied principal components analysis (PCA) to model weights from individual subjects and to combined data. This procedure produces a low-dimensional semantic space in which concepts that are represented similarly in the brain are close together but concepts that are represented differently in the brain are far apart. The resulting semantic spaces are similar across subjects, suggesting that different individuals share a common representational space for the meaning of speech. We then used the common semantic space to visualize the cortical organization of semantic processing in each subject. To show how the representation of each semantic dimension is distributed across cortex, we projected the model weights for each voxel onto each dimension of the semantic space, and then plotted voxel projections on the cortical surface of each subject. This process reveals highly complex semantic maps in temporal, parietal, and prefrontal cortices that are similar across subjects. In sum, our data suggest that different individuals share a common semantic space that is organized similarly across cortex.

Disclosures: A.G. Huth: None. **W.A. de Heer:** None. **F.E. Theunissen:** None. **T.L. Griffiths:** None. **J.L. Gallant:** None.

Poster

285. **Language II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.05/JJJ5

Topic: F.01. Human Cognition and Behavior

Title: Language lateralization in 297 healthy volunteers: Association with manual preference strength exists only in left-handers

Authors: *N. TZOURIO-MAZOYER, D. MARIE, L. ZAGO, G. JOBARD, E. MELLET, L. PETIT, F. CRIVELLO, M. JOLIOT, B. MAZOYER;
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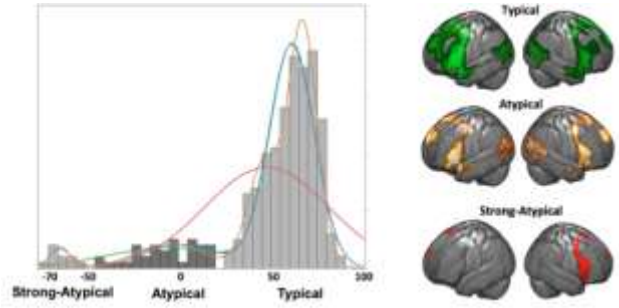
Abstract: 297 healthy volunteers (aged 25.3 years, [18, 57]), balanced for sex, and including 153 left-handers (LH), completed a sentence and word list covert production task (PROD) during BOLD fMRI. For each individual a left - right Hemispheric Functional Lateralization Index (HFLI) was computed using the LI toolbox for the contrast sentence minus word list production. Manual preference (MP) was quantified with the Edinburgh Score (right-handers (RH): 93.1 ± 11.0, LH: 60.0 ± 41.0). PROD HFLI distribution was optimally fitted by a mixture of 4 Gaussian functions mixture (figure) allowing the definition of 3 language Lateralization Types (LLatT): "Typical" HFLI > 18 (N=250), "Atypical" -50 > HFLI < 18 (N = 37), Strong-Atypical" HFLI < -50 (N = 10). Proportion of LH was lowest for Typical (48%), increased in Atypical (62%) and reached 100% for Strong-Atypical. Concordance between LLaT and hand lateralization (defined on a 3-level scale, Strong LH, weak MP, Strong RH) was not better than expected by chance alone (Kappa-statistic = 0.025, p = 0.17). In addition, while a logistic regression revealed a significant association between ES and the proportion of atypical subjects during PROD (p = 0.0001, atypicality occurrence increasing as ES decreases), such association was significant only in LH (RH: p = 0.56, LH: p = 0.011). Moreover HFLI did not differ between LH and RH in either the Typical (p = 0.31) or Atypical (p = 0.26) subgroups. Comparison of the functional anatomy of PROD between LatT groups revealed that Strong-Atypical and Atypical activated right hemisphere areas mirroring those more activated by Typical in the left hemisphere (figure). Between groups differences corresponded to differences in the left-right balance of frontal and temporo-parietal language areas. In conclusion, hand/language lateralization strength correlation exists in LH only. Right hemisphere lateralization for language is a rare (0.08% of the general

population), but normal, variant of language lateralization in healthy subjects.



Disclosures: N. Tzourio-mazoyer: None. **D. Marie:** None. **L. Zago:** None. **G. Jobard:** None. **E. Mellet:** None. **L. Petit:** None. **F. Crivello:** None. **M. Joliot:** None. **B. Mazoyer:** None.

Poster

285. **Language II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.06/JJJ6

Topic: F.01. Human Cognition and Behavior

Support: WT074414MA

Title: The perception of speech in noise and other maskers by musicians and non-musicians

Authors: *D. BOEBINGER[1], S. EVANS[1], C. LIMA[1], N. LAVAN[1], S. ROSEN[2], S. K. SCOTT[1];
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Abstract: Extensive musical training is associated with better speech-in-noise perception. However, here are multiple ways to mask speech with noise, and these various maskers show different behavioural and neural effects. Given that musical experience also seems to offset the age-related decline in speech-in-noise perception, understanding the relationship between musical experience and speech-in-noise perception is of great importance. This experiment uses fMRI and multiple types of speech maskers to determine whether there are differences in speech-in-noise perception between trained musicians and non-musicians, and whether that difference varies across masker types.
During the behavioural task, musicians (n=16) and non-musicians (n=16) passively listen to stimuli that consist of simple sentences masked with continuous noise, speech-modulated noise,

rotated speech, or another speaker. After hearing each sentence, the subject repeats as much as possible of the sentence. Subjects will be scored based on how many key words they get correct. All subjects will also fill out a questionnaire detailing their musical experience and complete tasks measuring their pitch discrimination, executive control, working memory, and nonverbal IQ. In the fMRI task, subjects listen to stimuli consisting of short narratives taken from a British newspaper and masked with either continuous speech, discontinuous speech, rotated speech, speech modulated noise, and continuous noise. Subjects are instructed to report as much as possible from the last phrase of the target speaker. Scoring is based on how many key words subjects correctly report. To determine whether performance differs between the musician and non-musician groups and between conditions, a musician x condition repeated-measures ANOVA will be performed for both the behavioural and fMRI phases of the experiment.
A behavioural advantage shown by musicians, relative to non-musicians, will replicate previous studies and extend these findings to include several types of maskers. A difference between the groups only with certain maskers could help determine the mechanism by which musicians are better able to perceive the target stimuli. Although the direction of causality cannot be inferred from this experiment, results could provide further evidence that musical training improves performance on certain linguistic and attentional tasks, as well as help clarify the underpinning neural systems.

Disclosures: D. Boebinger: None. **S. Evans:** None. **C. Lima:** None. **N. Lavan:** None. **S. Rosen:** None. **S.K. Scott:** None.

Poster

285. **Language II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.07/JJJ7

Topic: F.01. Human Cognition and Behavior

Support: EBRAMUS ITN 238157

Title: Neural correlates of contextual variability in second language learning

Authors: *L. VERGA[1], S. A. KOTZ[1,2];
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Abstract: Introduction
Learning new words firstly requires the understanding of word meaning. To accomplish this task, adult learners rely on cues provided by the context, in which a new word is encountered (1, 2). However, when a new word is repeated several times, different contextual cues can be used to

identify its meaning (3). The aim of the current study is to clarify the extent to which contextual variability influences the brain mechanisms underlying word learning in adults. We expect to observe both behavioural and neural differences during the learning phase as a function of contextual variability.

Methods

41 participants (20 F, mean age: 26 years, sd: 3.35 years) learned 20 new pseudowords during a scanning session (3T Siemens Trio scanner). In a given context, participants had to identify a matching object to which a pseudoword was assigned. Each object was repeated 9 times either i) in the same sentence context, or ii) in a different sentence context. A behavioural testing phase was subsequently conducted. Participants were presented with a novel context sentence. Their task was to select from 3 possible pseudowords the correct missing word learnt during the learning phase.

Results

Behaviourally, we observed an advantage for new words when embedded in the same sentence context as compared to a different context in terms of faster response times ($p<.001$) and greater accuracy ($p<.001$) during the learning phase. No differences were observed between the two conditions in the subsequent testing phase. At the neural level, the two conditions led to different activations in the left inferior frontal gyrus (BA 44) and middle occipital gyrus/inferior parietal cortex as well as the right cerebellum (all $p<.05$, FWE corrected for multiple comparisons, $k>20$ voxels).

Conclusions

Our results suggest that context variability influences the encoding of new words in adult learners. Further studies are required to evaluate the potentially differential impact of different contextual factors (such as a social context) on word learning.

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2.Mestres-Missé, A. et al. J Cognitive Neurosci, 2008
3.Lohans, L.J. et al. J Mem Lang, 2011

Disclosures: L. Verga: None. **S.A. Kotz:** None.

Poster

285. **Language II**

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NIH 5R21HD050558

NSF Grant SBE-0541953

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Title: Does early exposure to a visual signed language "hurt" auditory language tissue development: Evidence from fNIRS neuroimaging of language processing in deaf individuals cochlear implants

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Abstract: Controversy abounds regarding the specific impact of differences in language experience on the neural systems underlying auditory spoken language processing in deaf individuals with cochlear implants (CI). Does early exposure to a visual signed language impact classic spoken language tissue development, including left Inferior Frontal Gyrus (LIFG) and Superior Temporal Gyrus (STG), in the CI individual? We further investigate how language processing in the deaf CI individual is impacted by variation in early-life language experience. To address this question, two hypotheses are tested: (1) Early language experience, signed or spoken, facilitates normal neural development supporting language processing in the CI individual. (2) Only early spoken language experience facilitates normal neural development supporting language processing. Early signed language experience disrupts classic language tissue recruitment in the CI individual.

METHODS CI individuals (mean age of cochlear implantation: 13 y) with early (birth-5y) or late (5y+) exposure to a visual signed language read aloud single English words while undergoing functional near infrared spectroscopy neuroimaging (Hitachi ETG 4000). Like fMRI, fNIRS measures hemodynamics, but has key advantages for studying language: tolerates movement, nearly silent, has good spatial and greater temporal resolution (10Hz), and crucially, is safe for CI individuals (Shalinsky et al., 2009).

RESULTS The age of exposure to a visual signed language yielded differences in patterns of neural activation during auditory language processing. CI individuals with early signed language exposure showed greater activation in the LIFG during reading, whereas individuals with later signed language exposure showed greater activation in the right IFG.

The results provide a new view on how early life language exposure, irrespective of modality (signed, spoken), can facilitate language processing in the CI individual. The finding that late

exposed CI individuals showed greater activation in the right hemisphere, not in primary left hemisphere language tissue supports the hypothesis that early speech or sign exposure facilitates normal language processing. In contrast to the late exposed individuals, early signed language exposure facilitates typical LIFG activation. We found no evidence of a negative impact on language processing in CI individuals as a result of early visual signed language exposure. The findings have vital implications regarding the optimal developmental timing of signed language exposure: Early language exposure, be it signed or spoken, supports healthy, typical language development.

Disclosures: K.K. Jasinska: None. **C. Langdon:** None. **L. Petitto:** None.

Poster

285. **Language II**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.09/JJJ9

Topic: F.01. Human Cognition and Behavior

Title: Differential activation patterns in visual and motor areas after enriched foreign language learning

Authors: *K. M. MAYER[1], I. B. YILDIZ[1,2], M. MACEDONIA[1,3], K. VON KRIEGSTEIN[1]; [1]MPRG Neural correlates of human communication, Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Dept. d'Etudes Cognitives, Col. de France, Paris, France; [3]Johannes Kepler Univ. Linz, Linz, Austria

Abstract: Learning foreign languages in adulthood is time consuming and effortful. In education settings, learning is often based on verbal input such as hearing the language and reading text. Previous studies showed that enriching foreign language learning material with additional visual or motor information (e.g. viewing pictures or conducting pantomimic gestures) leads to significantly better learning outcome than exclusively verbal learning. It is unclear how enrichment can improve learning performance. At present, there are two theories explaining the enrichment benefits at the brain level. One attributes the enrichment benefits to increased activation in brain areas specialized for semantic processing. The other one attributes the enrichment benefits to recruitment of brain areas that are specialized in processing the provided enrichment. We conducted two behavioral and two fMRI studies to adjudicate between the two theories. We taught adults vocabulary in an artificial language with exclusively verbal and enriched learning material. We monitored learning progress with vocabulary tests. Furthermore, we conducted a vocabulary test during fMRI after vocabulary learning was completed.

Behaviorally, we found beneficial effects of both motor and visual enrichment. Two and six months after learning participants recalled significantly more words learned with self-performed gestures and pictures than words learned verbally or with other types of enrichment. At the brain level, multivariate pattern classifiers revealed that visual and motor areas carry information about the enrichment conditions during learning. Our findings support the theory that attributes enrichment effects to enhanced activation in areas specialized in processing enrichment.

Disclosures: K.M. Mayer: None. **I.B. Yildiz:** None. **M. Macedonia:** None. **K. von Kriegstein:** None.

Poster

285. **Language II**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.10/JJJ10

Topic: F.01. Human Cognition and Behavior

Support: German Science Foundation FOR-499

Title: Identifying hub structures of emotional speech in the human brain

Authors: *S. A. KOTZ[1], S. K. SCOTT[2], S. ROSEN[3], J. OBLESER[4];
[1]Neuropsychology, Max-Planck Inst. Cog & Brain Sci., Leipzig, Germany; [2]Insitute of Cognitive Neurosci., [3]Speech, Hearing & Phonetic Sci., UCL, London, United Kingdom; [4]Auditory Cognition, MPI for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Two distinct dimensions convey emotional content of speech: A verbal (segmental) “what” dimension and a non-verbal (prosodic) “how” dimension. As these two dimensions occur in tandem, it necessitates core structures in the brain to fuse them. By now, an extended brain network subserving emotional speech has been suggested1,2,3. However, controversy persists as to (i) which of these brain areas are driven by the “what“ and “how” aspects of emotional speech, and (ii) which candidate areas support their integration. Relevant hub structures of emotional speech should therefore not only be sensitive to the respective dimensions, but should also exhibit joint sensitivity to both. We applied acoustic manipulations previously employed in speech intelligibility research4 to dissociate segmental from prosodic information. These manipulations entailed rotated speech5 and vocoded speech6 next to intelligible speech. This allows identifying brain areas that dissociate and or fuse the “what” and “how” dimensions of emotional speech, with specific emphasis on the anterior superior temporal gyrus/sulcus (STG/STS) featuring prominently as a hub of emotional speech integrating the “what” and “how” information.

Methods
In 3-T (Bruker) functional magnetic resonance imaging (fMRI) using temporally sparse sampling, normal hearing participants (N=16, 8 female) passively listened to German sentences spoken with emotional and neutral prosody in a pseudo-randomized order of acoustic degradation formats (intelligible, rotated, noise-vocoded, and rotated+noise-vocoded).
Results
Contrasting emotional > neutral speech for (1) intelligible-vocoded speech and (2) intelligible-rotated speech, a conjunction analysis revealed hub structures in the bilateral anterior STS , the right ventrolateral orbitofrontal cortex (OFC), the cerebellum, and the left parahippocampal gyrus. We confirm a clear distinction of "how" (STG) and "what" (STS) dimensions of emotional speech bilaterally.
Conclusions
The application of acoustic manipulations allowed identifying hub areas of emotional speech. Next to these core areas, distinct fingerprints of "what" and "how" information were identified. The current results confirm recent evidence on emotional speech2 and emphasize the importance of the anterior temporal cortex, an area of recent controversy2,7 in emotional speech.
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Disclosures: S.A. Kotz: None. **S.K. Scott:** None. **S. Rosen:** None. **J. Obleser:** None.

Poster

285. **Language II**

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Program#/Poster#: 285.11/JJJ11

Topic: F.01. Human Cognition and Behavior

Support: NIH AG17586

NIH AG11516

NIH NS53488

NIH NS44266

NIH AG38490

Title: Selective grammatical comprehension deficits in non-fluent/agrammatic primary progressive aphasia

Authors: D. CHARLES, C. OLM, J. POWERS, S. ASH, D. J. IRWIN, C. T. MCMILLAN, K. RASCOVSKY, *M. GROSSMAN;
Dept Neurol., Univ. Pennsylvania Sch. Med., PHILADELPHIA, PA

Abstract: Grammatical comprehension difficulty is a necessary characteristic of the non-fluent/agrammatic variant of primary progressive aphasia (naPPA), also known as progressive non-fluent aphasia. However, clinical measures of grammatical comprehension are few, and those available are controversial. We developed a novel, two-alternative, forced-choice sentence-picture matching task sensitive and specific for grammatical comprehension, and examined this comparatively in 39 PPA patients (naPPA=12, logopenic variant PPA (lvPPA)=15, and semantic variant PPA (svPPA)=12) 27 non-aphasic patients with behavioral-variant frontotemporal degeneration (bvFTD), and 12 healthy controls. We also assessed the neuroanatomic basis for grammatical comprehension deficits in a subset of these patients with volumetric grey matter (GM) atrophy and whole-brain fractional anisotropy (FA) in white matter (WM) tracts. We found that patients with naPPA have selective difficulty understanding cleft sentence structures (e.g. “It was girls that boys chased”), while all PPA variants and bvFTD patients were impaired with more complex, center-embedded sentences (e.g. “Girls that boys chased were tall”). All patients had more difficulty with object-relative than subject-relative sentences. We also found that bvFTD patients are selectively impaired understanding sentences involving a strategically-placed adjectival phrase stressing short-term memory. Regression analyses related grammatical comprehension difficulty in naPPA to left anterior-superior temporal GM atrophy and reduced WM FA in anterior corpus callosum and inferior frontal-occipital fasciculus. Difficulty with center-embedded sentences in other PPA variants was related to other brain regions implicated in a large-scale sentence processing network. These findings emphasize a distinct grammatical comprehension deficit in naPPA, and associate this with interruption of a frontal-temporal neural network involved in sentence processing.

Disclosures: D. Charles: None. **M. Grossman:** None. **C. Olm:** None. **J. Powers:** None. **S. Ash:** None. **D.J. Irwin:** None. **C.T. McMillan:** None. **K. Rascovsky:** None.

Poster

285. **Language II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.12/JJJ12

Topic: F.01. Human Cognition and Behavior

Support: NSC 96-2628-H-002-073-MY2, National Science Council, Taiwan

MOE 2011-T2-1-031, Tier 2 AcRF, Ministry of Education, Singapore

Title: Modulation of phonological selection on interhemispheric connectivity

Authors: *C.-Y. WU[1], M.-H. R. HO[1], K. MATSUO[2], W.-Y. I. TSENG[2], C.-W. HUE[3], S.-H. A. CHEN[1];
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Abstract: Although language is known as a lateralized function, much research has reported bilateral recruitment of the frontal, parietal, and ventral occipito-temporal regions during Chinese phonological processing. We used functional magnetic resonance imaging with dynamic causal modeling (DCM) to examine interhemispheric connectivity within the bilateral language network. Thirty right-handed Chinese speakers (15 males; age M = 22.5, SD = 2.76) performed an event-related homophone judgment task on pairs of characters - many-to-one pairs (MO: characters with more than one sound) or one-to-one pairs (OO: characters with one sound) - in a 3T scanner. Image analyses were performed using SPM8 and DCM10. Only homophonic pairs (i.e., MO_H and OO_H) were considered in the current analyses. Volumes-of-interest were selected from 8 regions: inferior frontal gyrus (IFG), middle frontal gyrus (MFG), inferior parietal lobule (IPL), and fusiform gyrus (FG) bilaterally. The optimal model was determined from 16 models using the random-effects family-level Bayesian model selection method in a hierarchical structure with 4 levels. At each level, 4 models with different interhemispheric modulatory connections between each pair of bilateral FG, IFG, MFG, and IPL were compared sequentially. MO_H was found to recruit more activation than OO_H in the right MFG, and bilateral IFG and IPL (Fig. 1A), which could be attributed to higher loadings of orthography-to-phonology transformation, phonological selection and verbal working memory taxed by MO pairs. Within the selected model, however, only OO_H but not MO_H had significant modulations on the intra- and interhemispheric connections among IFG, MFG, and IPL (as illustrated in Fig. 1B). The results showed that reading MO_H pairs was associated with increased regional activity but reduced intrinsic couplings between activated regions. This may suggest that while the distributed network of activity may serve as the baseline for phonological processing, regional activity is enhanced and tends to work more independently as task demands increase.

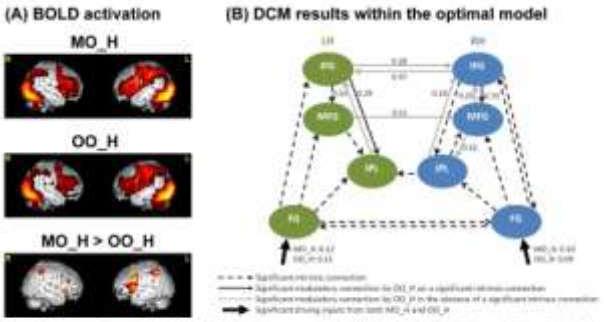

Figure 1. Summary of results. N = 30. (A) Group activation maps of the MO_H and OO_H conditions and the contrast of MO_H > OO_H, p < .005 (uncorrected). MO_H: many-to-one homophonic pairs, R and L: view at the right and left hemispheres, respectively. (B) The significant intrinsic connections, modulatory effects, and driving inputs within the optimal model selected using the family-level Bayesian model selection method in a hierarchical structure (p < .05). Significant intrinsic connections were all positive. Values represent the average parameter estimates (in Hertz) of the significant modulations and driving inputs. IFG: inferior frontal gyrus; MFG: middle frontal gyrus; IPL: inferior parietal lobule; FG: fusiform gyrus. Green regions are in the left hemisphere (LH) and blue in the right hemisphere (RH).

Disclosures: C. Wu: None. **M.R. Ho:** None. **K. Matsuo:** None. **W.I. Tseng:** None. **C. Hue:** None. **S.A. Chen:** None.

Poster

285. **Language II**

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Program#/Poster#: 285.13/JJJ13

Topic: F.01. Human Cognition and Behavior

Support: JSPS Grant #22220003

JSPS Grant #23240036

Title: Onomatopoeias and mimetic words represent mirror system but generate different mental images: An fMRI study

Authors: *K. YAOI[1], T. MINAMOTO[2], M. OSAKA[2], N. OSAKA[1];
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Abstract: In our daily lives, we often use a variety of onomatopoeias (hearing-based) and mimetic (visual movement-based) words. By using these words, we can enrich our expression during conversation and tell others easier our conscious quality by mirroring external world. Recently, some neuroimaging studies have investigated neural basis of onomatopoeias and mimetic words (Osaka et al. 2003), but there have been a few studies observing brain activation during image generation evoked by the words while keeping their eyes closed. Onomatopoeia and mimetic words are the unique exception to a rule of language theory that indicates no direct

link between word's sound and meaning as Saussure (1916) argued. Here we show that they have a direct link between sound and meaning by providing mirror neuron-based brain imaging evidences. Furthermore, we hypothesized that onomatopoeia and mimetic word could be dissociated by showing an independent brain activation areas evoked by onomatopoeia and mimetic words each other. In MRI scanner, 15 participants were instructed to listen Japanese onomatopoeias ("sawa-sawa" (sound of leaves flowing together), "kata-kata" (rattling sound), "hyu-hyu" (sound of wind blowing), "byu-byu" (sound of strong wind blowing), "wan-wan" (barking of a dog) and "nya-nya" (cat mewing)), mimetic words ("yochi-yochi" (baby toddle), "yota-yota" (walk awkwardly), "yobo-yobo" (elderly's infirm walk), "noso-noso" (walk sluggishly), "sui-sui" (move unrestricted) and "suta-suta" (walk briskly)) or no-meaning words ("heyu-heyu", "mepe-mepe", and so on). Then, they were asked to imagine visual or auditory image for each word. fMRI results indicated that onomatopoeias showed greater activation in left inferior frontal gyrus (IFG), left middle temporal gyrus, left premotor cortex and left parahippocampal gyrus than no-meaning words. Mimetic words also indicated greater activation in bilateral IFG, bilateral temporo-parietal junction, premotor cortex, supplementary motor area and some other regions than no-meaning words. These greater activations of IFG and premotor cortex were probably indicated that both onomatopoeias and mimetic words evoked mirror neuron systems. Then, from the direct comparison between onomatopoeias and mimetic words, mimetic words evoked greater activation in mid-cingulate cortex and medial prefrontal cortex than onomatopoeias, whereas onomatopoeias showed no greater activation. These results may indicate that onomatopoeias and mimetic words generate different types of mental image, and there are some neural dissociation between onomatopoeias and mimetic words.

Disclosures: K. Yaoi: None. **T. Minamoto:** None. **M. Osaka:** None. **N. Osaka:** None.

Poster

285. **Language II**

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Program#/Poster#: 285.14/JJJ14

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant HD057884-01A2

111 Project (B07008) from the Ministry of Education of China

NSF Grant BCS 0823624 and BCS 0823495

Title: Reading abilities in different languages are linked to variations in the same white matter microstructure

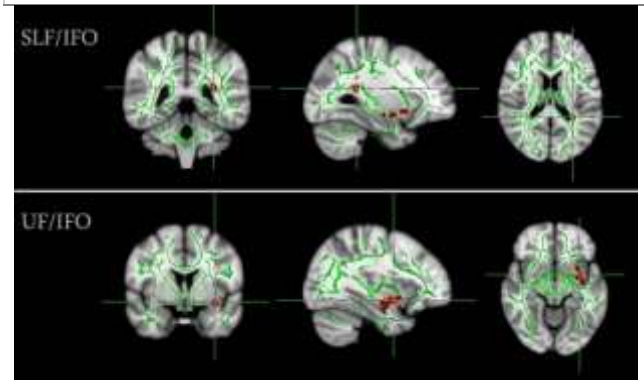
Authors: *M. ZHANG[1], C. CHEN[2], G. XUE[3], L. MEI[2], H. XUE[3], Q. DONG[3];
[1]Researching Backup Solutions, Beijing, China; [2]Univ. of California, Irvine, Irvine, CA; [3]Beijing Normal Univ., Beijing, China

Abstract: Recently, several studies discovered that white matter properties could be the efficient neural markers for reading abilities (Ben-Shachar et al., 2007; Friederici, 2009; Vandermosten et al., 2012). However, no study has evaluated the relationship between white matter connectivity and reading abilities across different languages. Using DTI, current study investigated the common and divergent relationship between white matter integrity indexed by fractional anisotropy (FA) and native language reading abilities.
95 students (age range: 18-30 years, mean age=20.8, SD=2.3, 56 female and 39 male) in U.S and 89 students (age range: 19-25 years, mean age=21.7, SD=1.7, 45 female and 44 male) in China were scanned. Their native language reading abilities were tested. Image processing was carried out by FSL (version 4.1.8) and TBSS (v1.2).
In both samples, white matter integrity in several fiber bundles correlated with their native reading abilities (Table1). Further conjunction analysis revealed the FA of two white matter fiber bundles in the left hemisphere was positively associated with native language reading abilities of both English and Chinese speakers: the inferior fronto-occipital fasciculus/superior longitudinal fasciculus (IFO/SLF) which is part of the dorsal phonological processing stream of reading, and the uncinate fasciculus (UF)/IFO which is part of the ventral semantic processing stream (fig1). The comparison between English and Chinese readers showed that no white matter integrity contributed only to either English or Chinese reading but not the other. These results support a common white matter microstructural basis for reading across language systems.

Table 1. Fiber clusters whose FA showed significant associations with reading abilities

Sample	Voxels in cluster	MNI coordinates of most significant voxel of cluster	Anatomical location of cluster	P value
U.S.	4543	-45, -30, -13	Corticospinal tract L	0.02
	1471	32, -25, -5	Inferior fronto-occipital fasciculus R	0.03
	52	-31, -19, 40	Superior longitudinal fasciculus L	0.05
	19	-9, 21, -10	Cingulum (cingulate gyrus) L	0.05
	7	-31, -16, 34	Superior longitudinal	0.05

			fasciculus L	
Chinese	3498	-15, -22, 31	Inferior fronto-occipital fasciculus L	0.02
	1098	26, -41, 25	Forceps major	0.03
	155	27, 5, 28	Superior longitudinal fasciculus R	0.04
	132	-13, -38, 38	Cingulum (cingulate gyrus)L	0.05
	104	20, -23, 49	Corticospinal tract R	0.05
	35	38, -38, 26	Superior longitudinal fasciculus R	0.05
	34	32, -85, 1	Inferior longitudinal fasciculus R	0.05
	32	32, -47, -3	Cingulum (hippocampus) R	0.05
	20	-9, -46, 43	NO label	0.05
	18	18, -89, -1	Forceps major	0.05
	8	18, -80, 3	Forceps major	0.05
	8	32, -38, -9	Inferior fronto-occipital fasciculus R	0.05



Disclosures: M. Zhang: None. **C. Chen:** None. **G. Xue:** None. **L. Mei:** None. **H. Xue:** None. **Q. Dong:** None.

Poster

285. **Language II**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.15/JJJ15

Topic: F.01. Human Cognition and Behavior

Support: NIH AG17586

NIH AG15116

NIH NS44266

NIH NS53488

NIH AG38490

NIH AG32953

Title: Silences in speech in primary progressive aphasia

Authors: *S. ASH, D. WEINBERG, J. HALEY, A. BOLLER, J. POWERS, C. MCMILLAN, M. GROSSMAN;
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Abstract: Efforts to characterize variants of primary progressive aphasia (PPA) on the basis of language-based clinical characteristics have met with difficulty. Nonfluent/agrammatic PPA (naPPA) has been described as “effortful,” although the nature of effortfulness is not well defined. These patients also have grammatical difficulty. Semantic variant PPA (svPPA) is distinguished by impairments of semantic memory and object knowledge, and they have difficulty with confrontation naming.
The present study quantifies the pattern of pauses in the speech of patients with PPA to determine how these pauses contribute to the apparent effortfulness of speech and how they relate to grammatical impairments and word-finding difficulty.
We studied 31 patients, including 18 with naPPA and 13 with svPPA, and 19 healthy seniors. A semi-structured speech sample was analyzed for pauses longer than 2.0 sec within utterances. We assessed the occurrence of these pauses in environments preceding or within a noun phrase (NP) and preceding or within a verb phrase (VP). Voxel-based morphometry was used to relate

gray matter atrophy to measures of performance.
naPPA patients produced long pauses more than other patient groups and paused in all environments indiscriminately, contributing to their slowed speech. Only naPPA had significant pauses related to verb phrases. svPPA patients paused more frequently in NPs compared to VPs. Pausing in the patient groups was related to differing cortical regions of the left hemisphere: to prefrontal and inferior temporal regions in naPPA and to temporal regions in svPPA.
The frequent occurrence of long pauses within utterances contributes to the effortfulness of speech in naPPA patients. Unlike the other groups, naPPA patients pause in the production of verb phrases as much as in the production of noun phrases, and this is linked to their grammatical difficulty. svPPA patients pause more in noun phrases than in verb phrases, which may be related to their semantic impairments. svPPA is relatively spared in verb phrase production as measured by long pauses.

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Poster

285. **Language II**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.16/JJJ16

Topic: F.01. Human Cognition and Behavior

Support: SNSF Grant # 138497

Title: Language context modulates reading route selection: An electrical neuroimaging study

Authors: *K. BUETLER[1], D. DE LEÓN RODRÍGUEZ[1], M. LAGANARO[2], R. MÜRI[3], L. SPIERER[1], J.-M. ANNONI[1];
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Abstract: Introduction: The Orthographic Depth Hypothesis (Katz & Feldman, 1983) posits that different reading routes are engaged depending on the type of grapheme/phoneme correspondence of the language being read. Shallow orthographies (e.g. German and Italian) with consistent grapheme/phoneme correspondences favor encoding via non-lexical pathways, where each phoneme is sequentially mapped to its corresponding grapheme. In contrast, deep orthographies (e.g. French and English) with inconsistent grapheme/phoneme correspondences favor lexical pathways, where phonemes are retrieved from specialized memory structures. This hypothesis, however, lacks compelling empirical support. The aim of the present study was to

investigate the impact of orthographic depth on reading route selection using a within-subject design.
Method: We presented the same pseudowords (PWs) to highly proficient bilinguals and manipulated the orthographic depth of PW reading by embedding them among two separated German or French language contexts, implicating respectively shallow or deep orthography. High density electroencephalography was recorded during the task.
Results: The topography of the event-related potentials to identical PWs differed 300-360 ms post-stimulus onset when the PWs were read in different orthographic depth context, indicating distinct brain networks engaged in reading during this time window. The brain sources underlying these topographic effects were located within left inferior frontal (German >French), left superior parietal (French > German) and left anterior cingular areas (German > French).
Conclusion: Reading in a shallow context favors non-lexical pathways, reflected in a stronger engagement of frontal phonological areas in the shallow versus the deep orthographic context. In contrast, reading PW in a deep orthographic context recruits "unnatural" non-lexical pathways, reflected in a stronger engagement of visuo-attentional parietal areas in the deep versus shallow orthographic context. These collective results support a modulation of reading route selection by orthographic depth.

Disclosures: K. Buetler: None. **D. de León Rodríguez:** None. **M. Laganaro:** None. **R. Müri:** None. **L. Spierer:** None. **J. Annoni:** None.

Poster

285. **Language II**

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Program#/Poster#: 285.17/JJJ17

Topic: F.01. Human Cognition and Behavior

Support: The Danish National Research Foundation's grant to CFIN

The MindLab grant from the Danish Ministry of Science, Technology and Innovation

Title: Phonemic versus allophonic status modulates early brain responses to language sounds: An MEG/ERF study

Authors: *A. H. NIELSEN[1,2], L. GEBAUER[1], W. B. MCGREGOR[2], M. WALLENTIN[1,3]; [1]Ctr. of Functionally Integrative Neurosci. (CFIN), [2]Dept. of Aesthetics and Communication - Linguistics, [3]Ctr. for Semiotics, Aarhus Univ., Aarhus C, Denmark

Abstract: Objective: An early component of the auditory event-related potential (ERP), the mismatch negativity (MMN/MMNm), has been shown to be sensitive to native versus non-native language sounds (Brandmeyer et al., 2012; Kazanina et al., 2006; Näätänen et al., 1997); i.e. sensitive to phonemic versus allophonic sound contrasts. So far this has only been attested between languages. In the present study we wished to investigate this effect within the same language: Does the same sound contrast that is phonemic in one environment, but allophonic in another, elicit different MMNm responses in native listeners?
For this purpose we employed the /t/-/d/-contrast in Danish (heavily aspirated versus unvoiced; only realized syllable-initially, not in syllable coda (Grønnum 2005)). Thus, [tæ] and [dæ] are separate words in Danish ('take' and 'then', respectively), whereas [æt] and [æd] are not (both meaning 'that').
This allowed us to manipulate the phonemic/allophonic status of exactly the same sound contrast (/t/-/d/) by presenting it in different immediate phonetic contexts (preceding a vowel (CV) versus following a vowel (VC)), in order to investigate the auditory event-related fields of native Danish listeners to a sound contrast that is both phonemic and allophonic within Danish.
Methods: Relevant syllables were recorded by a male native Danish speaker. The stimuli were then created by cross-splicing the sounds so that the same vowel [æ] was used for all syllables, and the same [t] and [d] were used for the relevant syllables. The final stimuli were validated by two native Danish listeners, ignorant to the design of the experiment. MEG was recorded from 17 native Danish listeners (5 females) while watching a silent movie and listening to the auditory stimuli. [tæ] and [æt] acted as standards, and [dæ] and [æd] thus as deviants, respectively. Data were preprocessed using Elekta's MaxFilter software and SPM8, all statistical analyses were conducted in sensor-space using SPM8.
Results: Focusing on the 150-300 ms time period after stimulus onset (typical MMNm time range for language sound contrasts), only the phonemic [tæ]-[dæ]-contrast showed significant effects (FWE-corrected at the cluster-level). Comparing the differences of the two contrasts, the phonemic [tæ]-[dæ]-contrast showed a significantly larger difference around 250 ms after stimulus onset than the allophonic [æt]-[æd]-contrast (FWE-corrected at the cluster-level).
Conclusion: By manipulating the immediate phonetic context, we demonstrate that native Danish listeners' early brain responses to exactly the same language sound contrast are modulated by its phonemic versus allophonic status.

Disclosures: A.H. Nielsen: None. **L. Gebauer:** None. **W.B. McGregor:** None. **M. Wallentin:** None.

Poster

285. Language II

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 285.18/JJJ18

Topic: F.01. Human Cognition and Behavior

Title: Role of posterior temporal lobe for language production revealed by transcranial magnetic stimulation study

Authors: *Y.-H. CHOI, H. PARK, N.-J. PAIK;
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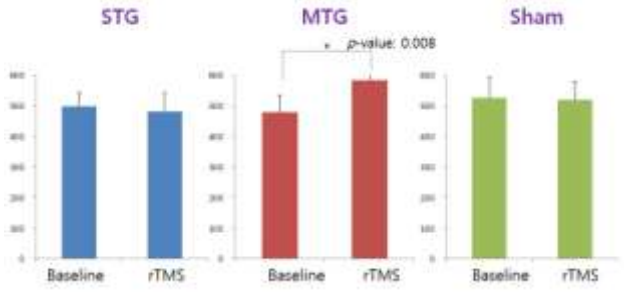
Abstract: Despite studies based on functional brain imaging suggest that superior temporal gyrus (STG) related pathway is engaged in phonological process, whereas middle temporal gyrus (MTG) related pathway in lexical-semantic one, the precise role of posterior temporal lobe is not sufficiently elucidated.
Neuro-navigation guided repetitive transcranial magnetic stimulation (rTMS) was used to identify the role of posterior temporal lobe during auditory language processing in the intact human brain and to clarify the functional relevance of the left posterior STG and MTG during auditory repetition and lexical decision tasks.
Twelve healthy volunteers were tested with lexical decision task (on word or pseudoword) and auditory repetition task (on word and sentence) prior to and during 3 sessions of rTMS or sham stimulation. We applied low-frequency rTMS to posterior STG and posterior MTG for 10 minutes in a random order. Reaction time and error rates for each task measured prior to and during stimulation were compared.
Stimulation of left posterior MTG significantly slowed response time of lexical decision task from 478.9±56.5 to 583.1±80.0 ms (p=0.008) (figure). However, stimulation over left posterior STG or MTG did not affect response time or error rates of auditory repetition task.
The present results suggest that left posterior MTG is engaged in lexical decision process. Neuro-navigation guided rTMS could be used to identify the functional role during language process.



Disclosures: **Y. Choi:** A. Employment/Salary (full or part-time):; Seoul National University Bundang Hospital. **H. Park:** A. Employment/Salary (full or part-time):; Seoul National University Bundang Hospital. **N. Paik:** A. Employment/Salary (full or part-time):; Seoul National University Bundang Hospital.

Poster

285. **Language II**

Location: Halls B-H

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Program#/Poster#: 285.19/JJJ19

Topic: F.01. Human Cognition and Behavior

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NSFC (31171073; 31222024; 31271115)

Fondazione Cassa di Risparmio di Trento e Rovereto

Title: Reading without speech sounds: VWFA and its connectivity in the congenitally deaf

Authors: X.-S. WANG[1], A. CARAMAZZA[2,3], M. V. PEELEN[3], Z. HAN[1], Y. BI[1];
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Abstract: The connections with language regions have been commonly assumed to contribute to the placement and development of the visual word form area (VWFA). In this study, we specifically examined the effects of connections with auditory speech regions in shaping VWFA by investigating the VWFA location distribution, activation strength, and its functional connectivity pattern in congenitally deaf participants. We found that the locations and activation strength of the VWFA in congenitally deaf participants were highly comparable to those of hearing controls. Furthermore, while the congenitally deaf group showed reduced resting-state functional connectivity between the VWFA and the auditory speech area in left anterior superior temporal gyrus, a similar intrinsic functional connectivity pattern between the VWFA and a fronto-parietal network was observed in the two groups. Together, these results suggest that auditory speech experience has consequences for aspects of the word form-speech sound correspondence network, but that such experience did not modulate VWFA's placement or response strength. Instead, the role of the VWFA might be to provide a representation that is suitable for mapping visual word forms onto language-specific gestures, be they speech or manual signs, without the need to construct an aural representation.

Disclosures: X. Wang: None. **A. Caramazza:** None. **M.V. Peelen:** None. **Z. Han:** None. **Y. Bi:** None.

Poster

285. **Language II**

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Support: NINDS NS21135

NINDS PO4813

NINDS NS40596

NINDS NS065120

NINDS NS07839601

Nielsen Corporation

NRSA MH0753170

Title: The role of high gamma power and theta- and alpha-phase coherence in brain network dynamics during language production

Authors: *J. C. CASE[1], E. CHANG[2], N. E. CRONE[3], J. PARVIZI[4,5], R. T. KNIGHT[1,6], A. Y. SHESTYUK[1];
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Abstract: Growing evidence suggests that the interaction between high- and low-frequency brain rhythms and within-frequency phase coherence among brain areas are essential mechanisms for network communication and information transfer among brain regions. Specifically, whereas neuronal activity within the high gamma range (70-150 Hz) has been shown to index local cortical activation, theta- (4-7 Hz) and alpha- (8-12 Hz) phase coherence across areas has been shown to reflect larger network dynamics. However, the interplay between these proposed mechanisms of neuronal information coding during cognitive tasks remains largely unexplored in humans. We examined the role of high gamma activation, as well as theta- and alpha-phase coherence during performance of two linguistic tasks with and without lexical

search (i.e., word repetition vs. word generation). We employed intracranial electrocorticography (ECoG), a unique technique that provides superior temporal resolution and reduced volume conduction. ECoG signals were recorded in 4 subjects with intractable epilepsy (3 left and 1 right fronto-temporal grids). Participants performed two tasks: in the word repetition task, patients were asked to repeat aurally presented words of varying lengths, and in the word generation task, patients had to generate a semantic antonym to each aurally presented word. The generation task was more cognitively demanding, as evidenced by more errors and non-completions as well as longer reaction times ($p < 0.01$). The generation task elicited greater increases in high gamma over the middle frontal and inferior frontal gyri, duration of which predicted reaction times ($p < 0.01$). Similarly, greater theta- and alpha-coherence between prefrontal and other brain regions, including temporal, motor, and parietal cortex, was evident in the generation relative to repetition task. Theta- and alpha-phase coherence was restricted to temporal and parietal areas in the repetition task. These findings suggest that high gamma activity and phase coherence support network communications among prefrontal, temporal, motor, and parietal areas during performance of complex linguistic tasks.

Disclosures: J.C. Case: None. **E. Chang:** None. **N.E. Crone:** None. **J. Parvizi:** None. **R.T. Knight:** None. **A.Y. Shestyuk:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 286.01/JJJ21

Topic: F.01. Human Cognition and Behavior

Support: Research Council of Norway, Drug Addiction Programme

Title: The μ-opioid system optimizes human reward behavior, a drift diffusion model account

Authors: *M. EIKEMO[1], G. BIELE[2], F. WILLOCH[3], S. LEKNES[2];
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Abstract: Balancing speed and accuracy of decisions, identifying the ‘best’ option when several rewards are available, and expending effort through enhancing attention to options, are all strategies for improving value based decisions. Computational models, like the drift diffusion model (DDM, Ratcliff & McKoon, 2008, Neural Comput), allow us to parse decision making into these constituents based on reaction time and accuracy data. The model permits us to estimate speed-accuracy trade-off (decision boundary, a), overall task performance (drift rate, v), and behavioral preference for one alternative (bias, b). This information can improve

understanding of reward processing in the human brain, and how reward behavior is optimized. While rodent studies show that the μ-opioid system is essential for several aspects of reward behavior, less is known about the role of the μ-opioid system for reward processing in the healthy human brain. Combining psychopharmacology and computational modeling, we investigated μ-opioid effects on reward behavior in 30 healthy, opioid naïve males. We predicted that μ-opioid receptor (MOR) agonism would enhance, and MOR antagonism would impair, reward behavior in humans. A two-alternative decision making task in which correct responses for one response alternative were more frequently rewarded (skewed reward schedule) was used. Three versions of the reward task were implemented using a randomized, placebo-controlled, double-blind cross-over design. Participants received a MOR agonist (morphine, 10mg), a non-selective opioid receptor antagonist with high MOR affinity (naltrexone, 50mg) or placebo on three separate days. Response data were analyzed using Bayesian estimation of DDM parameters (Wiecki et al., 2012, SfN abstract). Both the overall task performance (v) and preference for accuracy over speed (a) were higher in the agonist and lower in the antagonist condition compared to placebo. The overall preference for the most frequently rewarded response option (b) was higher in the agonist condition than in the placebo and antagonist conditions. These results suggest that the endogenous μ-opioid system guides reward behavior in humans by increasing motivation and attentiveness when rewards are available. The μ-opioid system may also influence the selection of the most valuable response option. Overall, our results confirm the hypothesis that the endogenous μ-opioid system optimizes human reward behavior.

Disclosures: M. Eikemo: None. **G. Biele:** None. **F. Willoch:** None. **S. Leknes:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 286.02/JJJ22

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1R01MH098899-01

Title: A drift-diffusion model of decision-making in dynamic environments

Authors: *C. M. GLAZE[1], J. I. GOLD[2];
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Abstract: Many kinds of decisions are based on evidence that is sampled sequentially over time to reduce uncertainty about the identity of the source of the evidence. Models of this kind of decision-making typically make the simplifying assumption that the source does not change

during the sampling process, making all sampled evidence relevant to determining the source. However, this assumption does not hold in real-world settings in which abrupt changes in the environment render past evidence irrelevant to future decisions. The goal of this study is to take well-established, sequential-sampling frameworks that have been applied to static, two-alternative forced-choice (2AFC) decision problems and extend them to account for dynamic environments that undergo such abrupt changes.

Our starting point is the drift-diffusion model (DDM), in which noisy evidence is accumulated over time to identify which of two possible sources generated the evidence. Here, we extend the model to account for abrupt change-points in the identity of the source that can occur during the decision process. The model is derived from the Bayes optimal solution and is related to estimation in a hidden Markov process. As in certain forms of DDM, the decision variable is the logarithm of the ratio of the conditional probabilities of the two options, given the accumulated evidence. The model forms sequential updates of this decision variable as a stochastic difference equation with a nonlinear term that accounts for a possibly time-varying generative process; i.e. a probability>0 that the "correct" decision will change at any given time (hazard rate). When the probability is zero, the nonlinear term drops out and the model reduces to classical DDM.

We support the model with pilot data from a 2AFC task in which human subjects decide which of two sources on a computer screen generated a given spatial data point on the current trial. After ~200-300 trials, subjects begin biasing their decisions by an amount that is proportional to the generative hazard rate, combining the trial-dependent prior probability of each source with the current likelihood in a nearly optimal manner. After correctly identifying change-points, subjects demonstrate immediate "flips" in the decision bias, the magnitude of which reflects the amount of certainty that a change-point occurred. This framework represents a new way to investigate how we form binary decisions in noisy, dynamic environments.

Disclosures: C.M. Glaze: None. **J.I. Gold:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant MOP-102662

CFI

FRSQ

EJLB Foundation

Fyssen Foundation

Title: Neural activity build-up during decision-making is not caused by evidence accumulation but by a growing urge to act

Authors: *P. E. CISEK, D. THURA;
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Abstract: Many studies, including extracellular recordings in monkeys and neuroimaging in humans, have reported that during decision-making tasks, neural activity builds-up at a rate related to the strength of sensory evidence. This is usually interpreted as the gradual accumulation of evidence to a decision threshold, often called the "bounded accumulation" or "drift diffusion" model. However, an alternative explanation exists: that sensory evidence is computed quickly and the neural activity build-up is instead due to a growing signal that reflects the urge to commit to an action. In most conditions previously tested, sensory information is constant over time, which renders these models mathematically identical and therefore indistinguishable. However, recent studies aimed at distinguishing these models (by presenting information that changes over time) strongly support the latter, "urgency-gating" model. Here, we present the mathematical foundation for why the urgency-gating model may be better suited to explain decision-making behavior in general. First, it implements a dropping criterion of accuracy, which achieves a higher reward rate than any setting of a constant criterion (as usually assumed by bounded accumulation). Second, it takes account of the fact that sequential samples are often partially redundant, and that estimates of evidence should primarily emphasize novel information. In simple tasks, an approximation of this optimal policy may be achieved by low-pass-filtering sensory input and then combining it with a growing urgency signal. We summarize behavioral and neural results of three studies which strongly suggest that sensory information is not integrated over time, but simply low-pass-filtered and combined with urgency. In summary, we suggest that despite the widespread acceptance of the bounded accumulation model, urgency-gating may offer a better explanation of decision-making mechanisms in a wide variety of tasks.

Disclosures: P.E. Cisek: None. **D. Thura:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Topic: F.01. Human Cognition and Behavior

Support: James S. McDonnell foundation

NIH T32

NIH R01

Title: Tracking the integration of time-varying sensory evidence with human EEG

Authors: *K. E. KAYE, E. F. ESTER, J. T. SERENCES;
UCSD, La Jolla, CA

Abstract: Theoretical and experimental work suggests that perceptual decision making is an integrative process: information about a noisy stimulus is continuously sampled and integrated until an internal criterion is reached. In a recent paper, O'Connell and colleagues (Nat Neurosci 15:1729-35) described a human ERP component - termed the centro-parietal-positivity, or CPP - that appears to track the temporal evolution of a decision variable in a gradual target detection task. However, it is unclear whether momentary changes in CPP amplitude track temporal fluctuations in the "strength" of sensory evidence, as would be expected of a decision variable. To examine this possibility, we recorded EEG while subjects viewed arrays of randomly oriented and spatially overlapping light and dark bars. During each trial, we gradually increased the percentage of light or dark bars that shared a common orientation (specifically, -45 or 45° with respect to vertical) up to an asymptotic limit (16-64% in increments of 16%), and subjects were instructed to report which set of bars (i.e., light or dark) shared a common orientation as quickly and accurately as possible. Critically, the percentage of iso-oriented bars in the target varied over the course of a trial. We reasoned that if the CPP reflects the temporal evolution of a decision variable, then momentary changes in the amplitude of this component should covary with changes in the strength of sensory evidence. Our data accord with this view. Specifically, we observed a large CPP component that reached a peak amplitude near the mean response latency for a given task difficulty level (i.e., 16-64%). Peak CPP amplitudes were substantially larger during correct relative to incorrect trials, but did not vary as a function of task difficulty. Critically, CPP slope increased monotonically with gradual increases in stimulus strength, consistent with the view that momentary fluctuations in the amplitude of this component reflect real-time changes in sensory evidence. These findings provide additional evidence suggesting that the CPP tracks the temporal evolution of a decision variable.

Disclosures: K.E. Kaye: None. **E.F. Ester:** None. **J.T. Serences:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

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Program#/Poster#: 286.05/JJJ25

Topic: F.01. Human Cognition and Behavior

Support: This paper is part of the Research Priority Program 'Brain & Cognition' at the University of Amsterdam.

Title: The role of the subthalamic nucleus in multiple alternative perceptual decision-making revealed by 7T structural and functional MRI

Authors: *M. C. KEUKEN[1,2], L. VAN MAANEN[1], R. BOGACZ[3], A. SCHAEFER[2], J. NEUMANN[2], R. TURNER[2], B. U. FORSTMANN[1,2];
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Abstract: In our everyday life we constantly have to make decisions between many different choice options. Recently, quantitative mathematical and neurocomputational models have been developed that make predictions about brain structures involved in decision-making with multiple alternatives. One such model is the Multiple Sequential Probability Ratio Test (MSPRT), which predicts that activity in the subthalamic nucleus (STh), a small structure in the basal ganglia, becomes more active with an increasing number of choice alternatives1,2. However, Frank et al. suggest that such an increase in STh activity might be caused by the amount of conflict between choice alternatives rather than the number of choices3.
The present study set out to test these two hypotheses using ultra-high 7T structural and functional magnetic resonance imaging in healthy human subjects. By simulating the MSPRT model, we generated specific predictions that allowed us to disentangle the influence of the number of alternatives and/or the amount of conflict between choice alternatives. These model predictions were then compared to the observed behavior as well as the activation pattern in the STh. Preliminary results are in line with the first hypothesis in that the activity in the STh is increased with more choice alternatives independent of the level of conflict.
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Disclosures: M.C. Keuken: None. **L. van Maanen:** None. **R. Bogacz:** None. **A. schaefer:** None. **J. Neumann:** None. **B.U. Forstmann:** None. **R. Turner:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 286.06/JJJ26

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1156601

Title: Perceptual decisions in the presence of relevant and irrelevant sensory evidence

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Abstract: In everyday life, perceptual decisions have to be made in the presence of both relevant as well as irrelevant sensory information. Thus, successful decisions require a separation of decision-relevant from irrelevant sensory evidence. To characterize how perceptual decisions are made in the presence of irrelevant sensory evidence, we asked human observers to make decisions about two-component random dot motion stimuli. On every trial, a fraction of the dots moved coherently along a horizontal axis, either leftward or rightward, while another fraction of the dots moved coherently along a vertical axis, either upward or downward. Only one of these two motion components was decision-relevant on any given trial, randomly selected and indicated by a cue at the beginning of the trial. Two choice targets were presented along a diagonal axis, and the subjects were instructed to make an eye movement to the target that was closest to the relevant direction of motion, while ignoring the irrelevant motion component. While our subjects were able to ignore the irrelevant information to a large degree, their choices and response times were still influenced by it, which allowed us to test computational models of perceptual decision making in the presence of irrelevant sensory evidence. The data pattern, including choices, mean response times, and the shape of response time distributions, is surprisingly well described by an integration-to-threshold mechanism for perceptual decisions between two alternatives, which is driven by a linear combination of the decision-relevant and irrelevant net sensory evidence signals. This suggests that the separation of decision-relevant from irrelevant sensory evidence can be characterized as a strong linear gain modulation.

Disclosures: J. Ditterich: None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

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Program#/Poster#: 286.07/JJJ27

Topic: F.01. Human Cognition and Behavior

Support: NIH 1 R01 MH098899-01

Title: Dynamic calibration of the influence of priors and sensory input for perceptual estimation

Authors: K. KRISHNAMURTHY, M. NASSAR, S. SARODE, J. CHEN, *J. I. GOLD;
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Abstract: Perception of an ambiguous sensory stimulus often depends on both sensory information and prior expectations about the presence or identity of the stimulus. Numerous studies support the idea that the brain follows Bayesian inference for a broad range of perceptual problems by combining each source of information in proportion to its reliability. However, this idea has typically been tested under conditions in which the reliability of prior expectations is relatively stable, which is often not the case in dynamic, real-world environments. More realistically, the underlying statistics of the environment can undergo changes that alter the reliability of prior expectations. Here we tested how abrupt, unexpected changes in the reliability of expectations about a stimulus affected the extent to which those expectations influence perception.

We used a novel sound-localization task to measure the influence of dynamically adjusted prior expectations on perceptual localization reports by human subjects. The location of a virtual sound source was varied randomly from trial-to-trial about a mean value, and on certain, randomly chosen trials, the location of the mean itself changed abruptly. On each trial, the subjects indicated both their prior expectation about the location of the sound before listening to the sound and the perceived location of the sound location afterwards.

We found that: 1) following a change-point in the virtual location of the sound source, when the prior reflected irrelevant data, the prior had the weakest influence on perceived location, and 2) on subsequent trials, both the reliability of the prior and its influence on perceived location increased steadily. These effects are consistent with an ideal-observer model describing the relative influence of priors and sensory evidence on perception in this environment. The results indicate that the brain is capable of rapidly adjusting the influence of bottom-up and top-down information on perception in a dynamic environment. Ongoing work is testing whether these effects are encoded by the pupil-linked arousal system, which we have shown can contribute to the updating of prior beliefs in a changing environment

Disclosures: K. Krishnamurthy: None. **J.I. Gold:** None. **M. Nassar:** None. **S. Sarode:** None. **J. Chen:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 286.08/JJJ28

Topic: F.01. Human Cognition and Behavior

Title: A rational analysis of sequential effects in perceptual decision-making: Fluctuations in RT and accuracy driven by prior bias due to recent trial history

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Abstract: Human subjects exhibit a sequential effect in many behavioral tasks: they respond more rapidly and accurately to stimuli that reinforce, rather than violate, a local pattern in stimulus history. For example, in 2-alternative forced choice (2AFC) tasks, despite a randomized design that deliberately de-correlate stimuli from trial to trial, subjects pick up transient sequences of repetitions and alternations; their responses are facilitated when a stimulus retains the pattern (e.g. AAAAA followed by A, ABABA followed by B), and are impeded when the stimulus violates the pattern (e.g. AAAAA followed by B, ABABA followed by A). Yu and Cohen (2009) used a normative Bayesian model, known as the dynamic belief model (DBM), to demonstrate that sequential effects may reflect adaptive learning in a changing environment. They showed that non-stationary prior belief can induce observed sequential effects in experiments, while using an otherwise Bayes-optimal algorithm. In this study, we examine the explicit consequence of a non-stationary beliefs on discrimination reaction time and accuracy in a 2AFC task. We model the relationship between a biased prior about stimulus type, generated by DBM, and speed/accuracy using a well-studied perceptual decision model, the drift-diffusion model (DDM). We jointly estimate the parameters for DBM and DDM in order to account for subjects' behavioral data, and show that the joint model accounts for trial-by-trial fluctuations in reaction time and accuracy due to recent trial history. Our result provide an explanation for the prevalence and persistence of sequential effects: the large behavioral benefit of being able to extract patterns when they exist versus the relatively small cost of finding them in error when the world is unpredictable.

Disclosures: S. Zhang: None. **A.J. Yu:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

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Program#/Poster#: 286.09/JJJ29

Topic: F.01. Human Cognition and Behavior

Support: NSF BCS0955037

Title: Brain activity during perceptual decision-making in the visual domain

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Abstract: The brain makes many moment-to-moment perceptual decisions, ranging from the identity of single objects to the relative timing of multiple external events, based on incoming sensory signals. How exactly does the brain integrate sensory information to arrive at these decisions? What are the brain mechanisms for making decisions when incoming sensory signals are weak or ambiguous? Using various perceptual categorization tasks at different difficulty levels in behavioral and fMRI experiments, we investigated the brain activity under the influence of ambiguity in object identity and motion directions from visual stimuli to answer these questions. In behavioral and fMRI experiments, thirty-three human volunteers performed visual categorization tasks, face-house and left -right motion discrimination. We found that the behavioral performance degraded and the reaction time increased with ambiguity or with task difficulty. A network of brain regions in the prefrontal and insular cortices showed elevated activity during difficult tasks and a network of early sensory visual regions showed elevated activity during easier tasks. We also evaluated how these regions and networks interact with each other. The differential brain activity in lower order sensory and higher-order decision-making brain regions and network activity between these networks provide us important clues about the functional organization of brain signals during perceptual decision-making processes.

Disclosures: B. Lamichhane: None. **B. Adhikari:** None. **M. Dhalama:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Topic: F.01. Human Cognition and Behavior

Support: ERC Advanced Grant FRONTEX

Title: Distortion of perceptual beliefs by categorical decisions in the human brain

Authors: *V. WYART, P. DOMENECH, J. DRUGOWITSCH, E. KOECHLIN;
Ecole Normale Superieure, Paris, France

Abstract: Real-life decisions need to be made and revised iteratively in face of an ever-changing environment. In the lab, however, perceptual decisions are usually studied independently from one another using paradigms in which the state of the environment changes abruptly following each decision. In such situations, perceptual decision-making has been described by near-optimal Bayesian inference, where individual decisions arise from probabilistic beliefs formed through gradual accumulation of sensory evidence. Past studies in primates have identified the neural substrate of this inference process in parietal and frontal cortices. Here we designed a categorization paradigm in which successive decisions were prompted at different points of a single, gradual inference process. Computational modeling of human behavior in this task revealed that, although decisions considered in isolation were indeed near-optimal, each of them triggered a specific distortion of the underlying perceptual belief: an inflated likelihood of the chosen category, and a reduced difference in perceived likelihood among unchosen categories. We analyzed magnetoencephalographic (MEG) and functional magnetic resonance imaging (fMRI) signals obtained from healthy human subjects engaged in this task, and isolated in space and time a functionally connected network of frontal and parietal regions responsible for this decision-triggered distortion of perceptual beliefs. We propose that this interaction between executive and perceptual systems allows for the compression of parametric, multi-dimensional representations of sensory information into their two first moments: a discrete, categorical percept, and a confidence level associated with it. By simplifying perceptual beliefs, this compression may also enable abstract, task-dependent rules to be applied to these beliefs, which is a hallmark of human reasoning and intelligent behavior.

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Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Title: Decision-making is influenced by a context-dependent urgency signal

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Abstract: Many models of decision-making propose that neural activity builds up to a threshold due to a gradual accumulation of sensory information. However, an alternative explanation is that sensory information is computed quickly, and the build-up in decision-related neural activity is due to a signal that grows over time, reflecting the urgency to make a choice. Distinguishing these models experimentally is difficult because they behave identically in most of the conditions previously tested. Here, we aim at distinguishing between them by presenting subjects with a Constant Motion Discrimination (CMD) task, in which subjects must decide on the direction of motion in a noisy random-dot motion display. Consistent with previous studies, we observed that brief motion pulses added to the stimulus have an effect on the subject's reaction times if presented early in the trial (<400ms) but not if they occur later. According to the bounded accumulation model, the reason late pulses do not have an effect is because the decision threshold has already been reached, and the decision has already been made. Following this reasoning, one would predict that if subjects adopt a slower decision policy by increasing the threshold, the effective window of pulses will expand to include both early and increasingly later pulses. In contrast, the urgency model predicts that as subjects adopt a slower decision policy the effective window for pulses will shift, rendering early pulses ineffective as later pulses become more effective.
We compared subjects' responses to motion pulses under two conditions; one in which CMD trials were blocked, and another in which identical CMD trials were interleaved among trials of a Variable Motion Discrimination (VMD) task in which the motion changes over time, leading subjects to wait longer before responding. We report three main results: First, analyses of the VMD trials showed that subjects' behavior was not consistent with bounded accumulation of the changing motion stimulus, but could be explained with the urgency gating model. Second, we found that average RTs in identical CMD trials were significantly slower when they were interleaved among VMD trials than when they were blocked together. Third, results to date suggest that the effective window for pulses in CMD trials shifts when they are interleaved among VMD trials, such that early pulses are no longer effective. These results can be accounted for by the urgency gating model, implemented as an attractor network whose dynamics are modulated by a growing urgency signal that is sensitive to broad task context.

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Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Program#/Poster#: 286.12/JJJ32

Topic: F.01. Human Cognition and Behavior

Title: Human learning and decision-making in a bandit setting exhibits forgetful Bayes and myopic planning

Authors: *A. J. YU, S. ZHANG;
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Abstract: We examine how humans actively explore a novel environment through repeated trials and noisy outcomes. We use a multi-armed bandit paradigm, where subjects choose one of four "arms" to pull on each trial and receive a binary outcome (win/lose). We compare human behavior to a variety of models that vary in their representational and computational complexity. Our result shows that subjects' choices, on a trial-to-trial basis, are best captured by a "forgetful" Bayesian iterative learning model (Yu & Cohen, 2009) in combination with a partially myopic decision policy known as Knowledge Gradient (Frazier, Powell, & Dayanik, 2008). This model accounts for subjects' trial-by-trial choice better than a number of other previously proposed models, including optimal Bayesian learning and risk minimization, epsilon-greedy, information maximization, and win-stay-lose-shift. It has the added benefit of being closest in performance to the optimal Bayesian model than all the other heuristic models that have the same computational complexity (all are significantly less complex than the optimal model). These results constitute an advancement in the theoretical understanding of how humans negotiate the tension between exploration and exploitation in a noisy, imperfectly known environment.

Disclosures: A.J. Yu: None. **S. Zhang:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Topic: F.01. Human Cognition and Behavior

Support: DFG LA2400/4-1

Title: Pre-stimulus alpha power biases perceptual decisions and confidences

Authors: *J. LANGE, T. BAUMGARTEN, A. SCHNITZLER;
Med. Faculty, Heinrich Heine Univ., Inst. of Clin. Neurosci. & Med. Psychology, Düsseldorf, Germany

Abstract: Previous studies have shown that perceptual decisions are based on the accumulation of sensory evidence, beginning at a starting point until a perceptual decision bound is reached. Less is known about decision making when sensory evidence is incomplete or ambiguous. Here, we studied decision making in a two-alternative forced-choice tactile discrimination task with ambiguous stimuli. We presented two brief electrical stimuli to 16 human subjects' left index finger with varying stimulus onset asynchrony (SOA) and subjects had to decide whether they perceived one or two stimuli. Additionally, they reported the subjective confidence of their decision. Individual SOAs were determined for which subjects reported to perceive one stimulus in ~50% of the trials (mean SOA: 25.9 ± 1.9). We used Magnetencephalography to study how inter-trial variability of subjective decisions is explained by fluctuations in ongoing and evoked brain activity.

Subjects made negligible errors for short SOA (0 ms, i.e. one stimulus) and long SOA (100 ms, i.e. two stimuli). For intermediate SOA (~25 ms), subjects perceived two stimuli in ~41% of all trials. Perception of two stimuli in trials with intermediate SOAs was predicted by significantly reduced alpha-band (8-12 Hz) power in contralateral somatosensory and posterior parietal regions -900 to -200 ms prior to stimulation. In addition, pre-stimulus alpha-band power predicted subjective confidence of the decision.

Trials with intermediate SOA and low pre-stimulus alpha-power showed significantly increased post-stimulus evoked fields (EF) relative to high power trials between 120-160 ms. Within this this time window , however, EF were highest when subjects unambiguously perceived two stimuli (SOA 100 ms) and lowest for unambiguously perceived one stimulus (SOA 0 ms).

We propose that the reported post-stimulus EF reflect a decision bound and pre-stimulus alpha-power reflects the (fluctuating) starting point of a decision process. Since the stimulation was short, sensory evidence could not be accumulated until the decision bound, reflected in variable decisions and lower confidence ratings. We suggest that this incomplete accumulation is influenced by pre-stimulus alpha-band power. Fluctuating alpha power shifts the decision variable towards either decision bound and thus spontaneously biases decisions.

Disclosures: J. Lange: None. **T. Baumgarten:** None. **A. Schnitzler:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Program#/Poster#: 286.14/JJJ34

Topic: F.01. Human Cognition and Behavior

Title: Practice in perceptual decision making produces structural changes in white and grey matter

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Abstract: Recent studies have shown that practice on cognitive tasks is accompanied by structural changes in the brain. However, it is still unclear which latent cognitive processes are involved in practice on cognitive tasks, and its underlying structural changes. In the current study, human participants were trained on a random-dot-motion task. Practice effects on performance were decomposed in latent cognitive processes by estimating parameters from the drift diffusion model (DDM). The grey and white matter structural changes associated with these parameter changes were examined using voxel-based morphometry (VBM) and tract-based spatial statistics (TBSS). The results showed that practice increases the rate with which participants extract information from the stimulus. This increase was associated both with increases in grey matter density and with an increase of white matter fractional anisotropy in a visuomotor network.

Disclosures: W. Boekel: None. **E. Wagenmakers:** None. **D. van Ravenzwaaij:** None. **B.U. Forstmann:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: CIHR MOP-97944

FRSQ Bourse de maitrise

Title: The impact of motion stimulus variability on the temporal dynamics of a target selection task

Authors: *E. LAM, J. F. KALASKA;
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Abstract: Random-dot kinetogram (RDK) stimuli with different degrees of coherent motion in one of two opposite directions (Britten et al 1996) have been widely used in behavioral and neural studies of decision-making about action choices. Data suggest that this decision process is driven by the net motion coherence and is highly stochastic, with possible sources of variability in both the RDK stimuli and central processing mechanisms.

We used two types of modified RDK stimuli that paired different amounts of coherent motion signals in both opposite directions. In the "narrow-coherence" (NC) set, the vector component in the coherent direction replaced the background Brownian motion of these dots for that frame. In the "Brownian-drift" (BD) set, the coherent vector component was added to their Brownian motion, rather than replacing it. For each set, different amounts of net unidirectional coherent motion (0 - 32%) were superimposed on different amounts of base coherence signal (0 - 32%) moving simultaneously in both directions, resulting in combinations of coherences ranging from (0%/0%) to (64%/32%).

We previously showed that subjects' performance (RTs and target choices) was influenced primarily by the net motion coherence across the range of tested base coherences (Lam & Kalaska SfN 2012). However, at low net coherences (0-8%), subjects' RTs tended to decrease as base coherence increased (cf., Niwa & Ditterich 2008). Total stimulus variance also increased with base coherence level. The present study examined to what degree the trial-to-trial variability of the observed visual RDK stimuli could explain the trial-to-trial variability in subjects' performance. Signal variability was measured both by counting the number of dots moving in each direction in each stimulus frame, and by using a motion energy filter analysis (Kiani et al. 2008).

For each stimulus combination, trials with shorter RTs had a greater variance of mean motion signal in the stimulus than trials with longer RTs. However, mean instantaneous stimulus variance was constant between short- and long-RT trials, indicating that shorter RTs did not result from inherently more variable stimuli. Similarly, there was no significant relation between the net directional deviation of the stimulus from the intended mean net coherence and either RTs or target choices. This evidence suggests that the stochastic variability in the RDK stimuli cannot account for the variability of subject performance. This in turn suggests that a central source of neural variability is a more important contributor to the stochastic nature of decision making process.

Disclosures: E. Lam: None. **J.F. Kalaska:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Topic: F.01. Human Cognition and Behavior

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Title: Modeling of subject independent and dependent cognitive biases in a resource allocation task

Authors: S. E. GOLDFARB[1], S. E. CHELIAN[1], J. D. COHEN[2], *R. BHATTACHARYYA[1]; [1]Information and Syst. Sci. Lab., HRL Laboratories, LLC, MALIBU, CA; [2]Dept. of Psychology and Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Cognitive biases such as loss aversion, risk aversion, and overreliance on exploration or exploitation cause suboptimal behavior in a variety of domains. For example, extreme loss sensitivity, in which subjects make overly conservative assessments after high loss decisions, has been offered as an explanation for probability matching, a suboptimal preference to match base rates to outcome probabilities as opposed to the Bayesian optimal strategy of binary assignments. Here we study cognitive biases in a variant of the n-armed bandit task in a military setting: given attack probability estimates for four groups, agents allocate troops to defend against a potential attack. An attack then occurs by one of the four groups and all troops not allocated against the actual attacker are said to be lost. In addition to finding subject independent probability matching, we further divide subjects into cognitive phenotypes that relate to dimensions of the Behavioral Inhibition System/Behavioral Activation System (BIS/BAS) scales. Using a recent neural network model that simulates interactions between neuromodulatory and prefrontal areas, we simulate these cognitive phenotypes by varying model parameters. Results suggest that we can account for subject independent and dependent cognitive biases by varying dimensions that can be derived from BIS/BAS questionnaires. Directions for future work include the addition of a control loop that may more closely approximate internal adaptive responses of decision makers who exhibit near-optimal performance, and/or may prove useful as an external (e.g., tutoring) mechanism for adaptively instructing those who exhibit suboptimal performance.

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Employment/Salary (full or part-time):; HRL Laboratories, LLC. 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.; IARPA. **J.D. Cohen:** A. Employment/Salary (full or part-time):; Princeton University. **R. Bhattacharyya:** A. Employment/Salary (full or part-time):; HRL Laboratories, LLC. 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.; IARPA.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Program#/Poster#: 286.17/JJJ37

Topic: F.01. Human Cognition and Behavior

Title: Optimal repeated change detection and value-based approximation

Authors: *T. TSUCHIDA[1], A. J. YU[2];
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Abstract: Sensory systems are often tasked with detecting discrete changes in the environment based on a noisy stream of inputs. Previously, we showed that the Bayes-optimal control policy for detecting the first change event in a single stream of noisy inputs requires accumulating Bayesian evidence up to a fixed threshold, and that the optimal procedure naturally gives rise to integrate-and-fire neuronal dynamics (Yu, NIPS, 2007). Here, we extend the framework to show that when the task is to detect repeated changes between signal-off and signal-on states, the optimal policy exhibits more complex dynamics than those of the LIF neuron models. In particular, penalizing duplicate detection signals induces refractory period profiles similar to what are observed in temporal receptive properties of early visual processing (Cai et al., J. Neurophysiol 78:1045-1061, 1997). The optimal change-detector requires maintaining and continually updating two state variables: the belief that world is in a signal-on state, and the onset of this state has not been previously detected. We explore whether this two-state solution can be efficiently and accurately approximated by a one-state representation that could be accomplished by a single neuron. We describe one such approximation, by representing the belief state jointly with the differential Q-values of the two possible actions (similar to q-factors in the Q-learning algorithm), the “detect” and “silent” actions, along with the time since the last output spike (detection event). The dynamics of this approximation is qualitatively similar to those of spike response models. We characterize how the properties of the optimal and

approximate change-detectors are altered by the behavioral task parameters, and we predict how the decision unit ought to adapt to these changes.

Disclosures: T. Tsuchida: None. **A.J. Yu:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

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Title: High gamma duration in human prefrontal cortex predicts categorical decision time

Authors: *M. HALLER[1], J. DEVRIES[2], N. J. HANSON[2], N. E. CRONE[3], E. F. CHANG[4], J. PARVIZI[5], R. T. KNIGHT[2,1], A. Y. SHESTYUK[1];
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Abstract: Categorization is a fundamental principal of cognition, allowing for the grouping of perceptually distinct objects and concepts. Categorical effects are observed across perceptual domains and involve decreased discrimination between members of the same category than between members of different categories. Converging evidence from human and animal studies indicates that the prefrontal cortex (PFC) plays a fundamental role in decision-making. In the current study we examined the spatio-temporal dynamics of categorical decision-making using electrocorticography (ECoG), which involves recording of neural signals directly from subdurally implanted electrode arrays in patients with intractable epilepsy (n= 14). Local field potential power in the high gamma band (70-150 Hz) has been linked to single unit activity and was used as an index of local cortical neuronal activity. Subjects performed several

categorization tasks, which used facial stimuli (emotion and gender categorization), adjectives (self-referential categorization), or morphed images or sounds (implicit and explicit perceptual categorization). Stimuli were presented either in the auditory or visual modality in each task. Local high gamma cortical activity in the middle and inferior frontal gyri (MFG, IFG) was evident in all categorization tasks, independent of sensory modality, and increased with task difficulty. Critically, the duration of the high gamma signal in PFC electrodes, but not in sensory regions, predicted the subjects' reaction time in all tasks (R range 0.44-0.74 across PFC electrodes, $p < 0.001$). The similarity of the high gamma duration effect across a diverse set of tasks demonstrates the central role of the PFC in categorical decision-making, spanning the spectrum from simple perceptual categorization to abstract categorical judgments. The results provide evidence that temporally integrated neural activity in the PFC supports decision-making.

Disclosures: M. Haller: None. **J. Devries:** None. **N.J. Hanson:** None. **N.E. Crone:** None. **E.F. Chang:** None. **J. Parvizi:** None. **R.T. Knight:** None. **A.Y. Shestyuk:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 286.19/JJJ39

Topic: F.01. Human Cognition and Behavior

Support: GRK 1589/1 "Sensory Computation in Neural Systems"

BMBF Grants 01GQ0414 and 01GQ1001

Title: The encoding of higher-order reward prediction errors during decision making

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Abstract: Humans are able to identify patterns in temporal sequences in order to predict future events. This kind of sequence learning requires not only to track the frequency of encountered stimuli, but also the higher-order statistical relationships between stimuli. In contrast, standard reinforcement learning models cannot accommodate this behavior, because they learn the mean expected future reward and bias action selection toward the most rewarding event. To explain how humans use the temporal structure of stimuli, we developed an adjusted Rescorla-Wagner (RW) model that learns the conditional probabilities of stimuli given the sequence of previous

stimuli. The validity of this model was tested in two experiments employing a two-armed bandit task, where a visual stimulus alternates between the left and right sides on the screen with varying statistical dependencies across blocks of trials. We utilized the Markovian steady state property to construct the stimulus sequences. In Experiment 1, we manipulated only the first-order conditional probabilities (e.g. the probability of the stimulus appearing twice in a row on the left) while in Experiment 2, second-order conditional probabilities were manipulated (e.g. the probability of the stimulus appearing on the left after a sequence of left and right). Importantly, all other lower-order conditional probabilities were fixed at 0.5 and the mean reward of each option was the same. 20 naïve subjects had to correctly predict the forthcoming stimulus location to gain a monetary reward. In both experiments, subjects' performance reached 80 percent correct when the stimulus alternated in a highly predictive manner. As expected, the RW model performed at chance when these conditional probabilities were smaller than 0.5, because the model could not capture the temporal dependencies of the task. In contrast, our adapted RW model was able to learn the conditional probabilities in the same manner as a standard RW model updates reward prediction. The model fitted the behavioral data significantly better than a random agent and closely simulated subjects' behavior. This indicates that conditional probabilities are estimated by higher-order reward prediction errors. We will investigate this hypothesis in detail by model-based fMRI to reveal the neural encoding of higher-order prediction error.

Disclosures: R. Guo: None. **K. Obermayer:** None. **M. Hebart:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 286.20/JJJ40

Topic: F.01. Human Cognition and Behavior

Support: Fondazione Cassa di Risparmio di Trento e Rovereto

Title: Distinguishing conceptual from motor decisions

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Abstract: We make decisions by giving more importance to one set of influences than to another set and deriving a choice from it. The predominant idea postulates that the brain accumulates evidence in a decision variable leading to some ramping activity, until the accumulated evidence

reaches a critical threshold to make a decision (1). Finally, the resulting choice can be expressed in many ways, by a motor act or by a mental activity, e.g. planning the upcoming holidays rather than tonight's dinner.
The fact that in several neurophysiological studies the same areas that show activity during motor planning also are activated in decision making has led to the view that decisions are made within the same sensorimotor circuits that are responsible for planning and executing the associated actions (2, 3). Such an intentional framework rejects the notion of conceptual decisions that are qualitatively distinct and separated from sensory and motor information.
We argue that decision making experiments that require overt reports, in particular with known SR mappings are confounding conceptual and motor decisions and may wrongly attribute functions to sensorimotor networks. We have attempted to disentangle perceptual decisions from motor decisions by successively presenting two stimuli (S1: face or house, S2: colored rectangle) and having participants perform a two step task in which a non-motor perceptual decision on S1 (face or house?) determined what participants had to overtly report about S2 (color or orientation). We monitored the blood oxygen level dependent (BOLD) response in 23 healthy participants with a 4T MR-scanner.
We found that the BOLD signal increased bilaterally in the ventral portion of precentral sulcus, and in the precuneus (areas 7 and 31) irrespective of whether participants were categorizing pictures, shapes or colors. Motor areas showed an increase in BOLD only when participants made overt responses.
Our results show that activity in motor areas reflects the motor decision (preparation and execution) but not the perceptual decision, while other areas reflect perceptual decisions abstracted from the input domain and the behavioral output. We argue for the notion that the brain represents conceptual decisions distinct from sensory and motor information.
1.M. N. Shadlen, W. T. Newsome, J Neurophysiol 86, 1916 (Oct, 2001).
2.P. Cisek, J. F. Kalaska, Annu Rev Neurosci 33, 269 (2010).
3.M. N. Shadlen, R. Kiani, T. D. Hanks, A. K. Churchland, in Better Than Conscious? Decision Making, the Human Mind, and Implications for Institutions, C. Engel, W. Singer, Eds. (MIT Press, Cambridge, MA, 2008), pp. 71-102.

Disclosures: J.V. Schwarzbach: None. **A. Caramazza:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 286.21/JJJ41

Topic: F.01. Human Cognition and Behavior

Support: Florida State University's "Big Questions in Free Will" initiative

Human Frontier Science Program

Weizmann Institute of Science – National Postdoctoral Award Program for Advancing Women in Science

Mathers foundation

Title: Dissecting different types of decision-making: An ERP study of reasoned vs. unreasoned voluntary decisions

Authors: *L. MUDRIK[1], U. MAOZ[1], D. XU[1], C. DUNCAN[1], Q. ZHANG[2], C. KOCH[1,3]; [1]Caltech, Pasadena, CA; [2]Natl. Univ. of Singapore, Singapore, Singapore; [3]Allan Inst. for Brain Sci., Seattle, WA

Abstract: The neuroscientific study of voluntary action suggests that consciousness may follow rather than initiate the decision to act. However, these experiments examined meaningless, arbitrary actions that bear no future consequences - as opposed to meaningful decisions that involve reasoning. Here, we used EEG to directly compare consequential, reasoned decisions (i.e., "choosing") with consequential, unreasoned decisions ("significant picking") and inconsequential, unreasoned decisions ("insignificant picking"). In Experiment 1, subjects pressed a button with their right or their left hand to either play against the computer in a matching-pennies game, where they could win or lose a fixed amount of money (choosing); guess whether that amount of money is associated with the left or right button (significant picking); or perform an arbitrary movement that always led to a gain of that amount (insignificant picking). Subjects were informed of the type of task at the beginning of each trial (stimulus onset). Choosing trials elicited a stronger centrofrontal positivity than both types of picking, starting as early as 300ms after stimulus onset, accompanied by a left posterior negativity. Further analysis revealed that these effects stem from differences in spatial distributions of brain potentials and not only in amplitudes. Interestingly, choosing trials also elicited a stronger central negativity that overlapped - yet not preceded - subjects' report of consciously deciding. In Experiment 2, we obtained similar results using a very different paradigm, based on food preference decisions. In each trial, two food brands were presented. Subjects again pressed right or left button to select one item (choosing), to guess a random association of the buttons either with both items or with two of their least favorite items (significant picking), or to perform an arbitrary movement that always resulted in receiving both items (insignificant picking). This paradigm required no tracking of choice history, nor involved competition against another player. Yet, choosing trials again gave rise to frontal positivity accompanied by posterior negativity that started at ~300ms. Taken together, our results suggest that decision type affects the very early stages of decision making, rather than the later ones that presumably lead to the conscious experience of deciding. Then, post-decision processes (e.g., result monitoring) are again modulated by decision type.

Disclosures: L. Mudrik: None. **U. Maoz:** None. **D. Xu:** None. **C. Duncan:** None. **Q. Zhang:** None. **C. Koch:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 286.22/JJJ42

Topic: F.01. Human Cognition and Behavior

Support: NSERC CREATE

CIHR

Title: A distributed functional network underlying strategic decision-making

Authors: *A. C. PARR[1], B. C. COE[1], D. P. MUNOZ[1], M. C. DORRIS[2,1];
[1]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada; [2]Inst. of Neurosci., Shanghai Inst. for Biol. Sciences, Chinese Acad. of Sci., Shanghai, China

Abstract: During competitive social interactions, one's chosen actions and their associated outcomes change dynamically based on the actions of other agents. This often requires that one avoid exploitation from opponents by employing the use of mixed-strategies; choosing among available actions unpredictably and stochastically. During strategic interactions, an individual's history of choices and reward outcomes, as well as those of their opponent, are used to predict future outcomes of each action by estimating the value of available actions. The neural mechanisms that calculate abstract value representations and transform these into specific actions during human decision-making are not well understood. Although game theory outlines mathematical frameworks to predict optimal strategies in social situations, discrepancies exist between these optimal strategies and how people actually behave.
The current study used functional MRI to investigate the neural processes related to mixed-strategy decision-making. A colour-based version of Matching Pennies was played against a dynamic computer opponent that exploited biases in players' response patterns. Participants selected one of two different coloured visual targets, and were rewarded if their selection matched that of the opponent. Results were contrasted with an instructed control task that matched the strategic task in terms of sensory input, choice direction, and reward rate. Therefore, any differences in brain response patterns should highlight strategic-related processes. Individuals behaved strategically, choosing each option in equal proportions, and unpredictably from trial-to-trial. More importantly, strategic decision-making was associated with activation of a highly distributed network, including bilateral dorsolateral prefrontal cortex, parietal cortex,

anterior cingulate gyrus, and the insular cortex. We propose that the observed pattern of brain activation represents the core elements of a distributed functional network underlying strategic forms of decision-making.

Disclosures: A.C. Parr: None. **B.C. Coe:** None. **D.P. Munoz:** None. **M.C. Dorris:** None.

Poster

286. **Decision Making: Drift Diffusion Models and Perception**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 286.23/JJJ43

Topic: F.01. Human Cognition and Behavior

Support: Ministry of Internal Affairs and Communications entitled, 'Novel and innovative R&D making use of brain structures'

Title: Hierarchical decision-making in humans: An fmri study

Authors: *W. YOSHIDA;
ATR, Kyoto, Japan

Abstract: Making optimal decisions requires the ability to select mappings between states and actions based on internally maintained representations of the environment. When the environment is large, hierarchical representation of state-space is effective, and computational models with a hierarchical extension are commonly used in engineering problems such as speech recognition and robot-navigation. Hierarchical organization can also be seen as a function of the structure of human behaviour, and humans build a hierarchical cognitive map that contains multiple levels representing the same large environment at different resolutions. Recent studies suggest that the prefrontal cortex represents an environmental model and has functionally hierarchical organization, with more anterior regions having increasingly abstract representations. However, the computational and brain mechanisms of decision-making in a hierarchical environment are still not fully understood.
To address this question, we designed a hierarchical maze navigation task in which two levels of state representation are required. First, the subjects are trained to learn the structures and the goal positions of three different types of maze. The mazes are partially-observable; the subjects infer their current positions from the observation of circum-spaces which are frequently ambiguous. After the training, they performed an experimental task in which they navigated in the learned maze to reach the goal but the type of maze (higher-level hidden state) and the current position in the maze (lower-level hidden state) were both unknown. Thus, to achieve this task efficiently required inference of two different levels of hidden states concurrently.

Nineteen healthy subjects performed the experiment in an fMRI scanner and we examined their brain (BOLD) activities related to their behaviours. We found strong reward-related brain activities in basal ganglia when the subjects reach the goal, and the activities of distinct regions in the parietal cortex with different spatial situations and navigation behaviours. We then use a computational technique based on probabilistic model inference: we statistically estimated the individual subjects' on-going state estimate and its associated uncertainty, based on their behavioural sequence, and then used this model to generate regression functions to predict brain activities. We compare models with and without a hierarchical environmental model and show the brain regions which are involved in hierarchical state inference in navigation.

Disclosures: W. Yoshida: None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.01/JJJ44

Topic: F.02. Animal Cognition and Behavior

Support: DSP support

Title: Comparing restoration of novel object recognition (NOR) in mice sub-chronically (sc) treated with NMDA receptor non-competitive antagonists phencyclidine (PCP), dizocilpine (MK-801), or ketamine, with rats treated with PCP: Effect of 5-HT_{1A} partial (PA) agonism and $GABA_A$ receptor stimulation

Authors: *L. RAJAGOPAL[1], Y. OYAMADA[1,2], M. HUANG[1], H. Y. MELTZER[1];
[1]Psychiatry and Behavioral Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL;
[2]Dainippon Sumitomo Pharma Co., Ltd.,, Osaka, Japan

Abstract: Introduction: Sub-chronic (sc) administration of NMDA receptor antagonists to rodents produce deficits in cognitive function, including novel object recognition (NOR), an analog of human declarative memory (DM). The hypoglutamatergic current produced by sc phencyclidine (PCP) has been considered to model the deficits in glutamate function in schizophrenia (Yuen et al.,2011). We and others have previously reported that atypical, but not typical antipsychotic drugs (APDs), can restore the deficit in NOR produced by sc PCP in female rats (Horiguchi et al., 2011) and that 5-HT_{1A} receptor partial agonism (PA), 5-HT_7 antagonism, and $GABA_A$ receptor stimulation can also reverse these deficits in female rats. We sought to determine if similar effects occurred in mice, as background for studies in transgenic mice, and whether the mechanisms effective in rats might also restore NOR following sc treatment with

two other NMDAR non-competitive antagonists, ketamine and dizocilpine (MK-801), in C57BL/6 mice. The novel APD, lurasidone (a D_2 antagonist/5-HT_{1A} PA,/5-HT_7 and 5-HT_{2A} antagonist properties) and a 5-HT_{1A} PA, tandospirone were tested in PCP, ketamine, and MK-801-treated mice. **Materials and methods:** PCP (10 mg/kg), MK-801 (0.1 mg/kg), and ketamine (30 mg/kg) were administered for 7 days followed by 7 days washout to male C57BL/6 mice. On the test day, day 15 or thereafter, acquisition trial was conducted for 10 min, in habituated mice, followed by a retention trial 24 hr later. Vehicle, lurasidone (0.3 mg/kg) or tandospirone (0.1 mg/kg) were administered 30 min before the acquisition trial. **Results:** Sc PCP, MK-801, or ketamine all induced deficits in NOR ($P<0.05$-0.01) compared to control mice. Lurasidone ($P<0.05$-0.001) or tandospirone ($P<0.05$-0.001 significantly reversed the NOR deficit in each group. The $GABA_A$ positive allosteric modulator, TPA023, and the 5-HT_7 antagonist SB269970 also reversed the NOR deficit in PCP-treated mice. **Discussion:** This study confirms that sc NMDAR antagonist treatment in mice produce a NOR deficit in mice, comparable to rats. The reversal of the NOR deficits produced by all three NMDAR treatments by lurasidone and tandosprione indicates a shared mechanism as a basis for the deficit and no obvious need to study more than one NMDAR antagonist. Thus, reversal of the scPCP-induced deficit with TPA023 and SB69970 is likely to also occur in the sc ketamine and sc MK-801-treated mice. However, in light of the development of intravenous ketamine for the treatment of severe depression, the cognitive deficit produced by sc ketamine may have special clinical relevance.

Disclosures: L. Rajagopal: None. **Y. Oyamada:** None. **M. Huang:** None. **H.Y. Meltzer:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.02/JJJ45

Topic: F.02. Animal Cognition and Behavior

Title: Scopolamine alters encoding but not retrieval on the E-maze version of the what/where/which task

Authors: *A. JOHNSON[1], C. STANCHFIELD[2];
[1]Psychology, Bethel Univ., MINNEAPOLIS, MN; [2]Biol., Bethel Univ., St. Paul, MN

Abstract: Novelty preference in the E-maze version of the what/where/which task is dependent on the hippocampus (Easton et al., 2009). We examined the development of novelty preference, vicarious trial and error behaviors, and pausing behavior at the choice point. Although a slight

novelty preference develops across training, the behavior that underlies novelty preference undergoes significant reorganization. Early in training, longer pausing at the choice point is associated with heightened novelty preference. Late in training, longer pausing at the choice point is associated with a preference for the familiar object.
We then used systemic scopolamine injections delivered immediately prior to an encoding session in order to assess the contribution of acetylcholine-based encoding processes to recollective memory and injections delivered immediately after an encoding session in order to assess the contributions of acetylcholine-based retrieval processes to recollective memory within the E-maze. Novelty preference was compromised by scopolamine during encoding and caused a shift to a preference for the familiar object in a subset of animals. Scopolamine treatment also induced a reversal in the relationship between novelty and time spent at the choice point in a trial-by-trial analysis. In contrast, scopolamine treatment rescued novelty preference in the retrieval condition - most likely as a result of reduced interference.
These results suggest that acetylcholine-based encoding contribute to recollection on the E-maze version of the what/where/which task while recollection-based retrieval is not acetylcholine dependent. These findings are consistent with a memory model in which familiarity and recollective memory processes share an encoding mechanism and only diverge during memory retrieval.

Disclosures: A. Johnson: None. **C. Stanchfield:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.03/JJJ46

Topic: F.02. Animal Cognition and Behavior

Title: Hippocampal activation of estradiol receptors α and β restores memory deficit caused by chronic estrogen deprivation

Authors: *C. P. BASTOS, L. M. PEREIRA, G. S. PEREIRA;
Physiol. and Biophysics, Federal Univ. of Minas Gerais, Belo Horizonte, Brazil

Abstract: We showed previously that object recognition memory is compromised by chronic estrogen deprivation in mice. Here we decided to test the hypothesis that the memory is impaired due the lack of estradiol modulation on hippocampal plasticity. To address this question, we used female C57BL6 mice that went to ovariectomy (OVX) at 8 weeks age. Eleven weeks latter, mice were submitted to stereotaxic surgery to implantation of bilateral cannulae in the dorsal

hippocampus (AP-1.7 mm from bregma; LL± 1.9 mm from midline; DV-1.0mm from the skull surface). After one week of recovery, mice were submitted to the object recognition task that consists of three phases: habituation, sample and choice phases. In the habituation, mice were placed in an empty plastic box and allowed to freely explore it for 5 min. Twenty-four hours later, in the sample phase, mice were first re-habituated to the empty box for 1 min. Next, two identical objects were placed on the right and left side of the box and the mice explored the objects for 30s in total. The choice phase was similar the sample phase except that a new object replaced a familiar one. Immediately and 3hs after the sample phase the animals received intra-hippocampal 0.5 µL/side of one the following drugs: vehicle (ciclodextrine or 50% DMSO), 17β-estradiol (E2, 5.0µg/µl), the ERα agonist propyl pyrazole triol (PPT, 1.0µg/µl) and the ERβ agonist diarylpropionitrile (DPN, 2.0µg/µl). Mice were tested in the choice phase 24 h after sample phase to evaluate the long-term memory (LTM). The preference for one object over another was assessed using one-sample t-test to determine whether the preference of the novel object differed significantly from hypothetical value 15. First, we demonstrated that intra-hippocampal infusion of E2 immediately after the sample phase but not 3hs after, increases the time exploring the novel object (15.38s ± 2.50s vs. 18.33s ± 3.01s, p = 0.04) and compared to vehicle group (15.62s ± 1.75s vs. 18.33s ± 3.01s, p = 0.04). Next, we showed that animals that received intra-hippocampal administration of either PPT (15.88s ± 2.58s vs. 18.80s ± 2.58s, p= 0.03) or DPN (15.62s ± 1.75s vs. 20.33s ± 3.83s, p= 0.01) spend more time with the novel object compared to vehicle group. Thus, our results showed that the reestablishment of E2 action on the hippocampus, right after learning, allows object memory to persists in chronic OVX mice. Furthermore, we showed that the activation of both ERα and ERβ improve the memory deficits related to chronic hormonal deprivation. Sources of research funding: Fapemig, CNPq, Capes.

Disclosures: C.P. Bastos: None. **L.M. Pereira:** None. **G.S. Pereira:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.04/JJJ47

Topic: F.02. Animal Cognition and Behavior

Support: National Natural Science Foundation of China 31121061

National Natural Science Foundation of China 30700218

Title: Methylphenidate acts within the prefrontal cortex to enhance memory storage

Authors: *S. LIU[1], F. YI[1], B. LI[1,2], X. ZHANG[1];
[1]Inst. of Neurobio., Fudan Univ., Shanghai, China; [2]Ctr. for Neuropsychiatric Dis., Nanchang Univ., Nanchang, China

Abstract: Methylphenidate is one of major treatments ameliorating cognitive dysfunction in attention deficit hyperactivity disorder (ADHD) and can improvement learning and memory. However, such improvements have previously been thought to result from increased attention. Here, we showed that methylphenidate directly alters mechanisms underlying learning and memory. In the present study, we investigate the effect of methylphenidate on synaptic potentiation and behavioural memory in the prefrontal cortex of young rats. Our results show that methylphenidate enhances long-term memory storage and facilitates long-term synaptic potentiation via dopaminergic D1 receptors and noradrenergic β1 receptors in the prefrontal cortex.

Disclosures: S. Liu: None. **F. Yi:** None. **B. Li:** None. **X. Zhang:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

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Program#/Poster#: 287.05/JJJ48

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant DA07625

NIH grant DA06634

DARPA N66001-09-C-2080

Title: Role of cannabinoid receptors and endocannabinoids in an animal model of Post-Traumatic Stress Disorder

Authors: W. D. JOHNSTON, *C. SEXTON, D. FETTERHOFF, A. J. SWEATT, S. A. DEADWYLER, R. E. HAMPSON;
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Abstract: Post-traumatic stress disorder (PTSD) is a growing problem in psychological health, especially among veterans, with patients exhibiting flashbacks, heightened anxiety, and impairment of memory and impulse control. PTSD is often associated with alcohol and drug abuse suggesting that neural systems affected by drugs also interact with stress mechanisms. An animal model of intense stress, the Single Prolonged Stress (SPS) procedure (Liberzon and

Young, Psychoneuroendocrinology, 1997; 22:443-453), has been demonstrated to cause physiological and behavioral changes seen in human patients, as well as increased glucocorticoid receptor expression in the hippocampus (Knox et al., Neuroscience 2012; 223:163-173). Moreover, deficiencies in cannabinoid receptor (CB1R) and endogenous cannabinoid signaling mimic the effects of chronic stress (Hill et al., Cerebral Cortex 2011; 21:2056-2064), suggesting an interaction of CB1R signaling in the hippocampus and PTSD-like effects on memory. Previous studies have successfully blocked some of the effects of SPS by administration of the CB1 agonist WIN 55,212-2 one day after the procedure (Ganon-Elazar and Akirav, Neuropsychopharmacology 2012; 37:456-466); therefore we hypothesize that effects of stress on hippocampal function may be modulated by cannabinoid receptor agonists or antagonists. We used performance on a short-term memory task, Delayed Nonmatch to Sample (DNMS), as a measure of hippocampal function. Adult male Long-Evans rats were exposed to SPS: (1) prior to training in the DNMS task; (2) after training, but prior to DNMS testing; and (3) prior to reversal to the Delayed-Match-to-Sample (DMS) contingency. SPS-treated rats exhibited delayed progression through the stages of DNMS training, and were impaired during DNMS-to-DMS reversal , but short term memory performance was unaffected at any length of delay. Comparison of post-SPS performance on DNMS training and reversal showed similarities to the effects of a cannabinoid agonist on these processes, suggesting that CB1Rs may mediate aspects of the effects of PTSD on learning and memory. Administration of a CB1R agonist shortly after an intense stressor may interfere with the development of changes in the endocannabinoid system which facilitates these effects. These results will be discussed with respect to potential therapeutic intervention for both PTSD and concomitant drug use

Disclosures: W.D. Johnston: None. **C. Sexton:** None. **D. Fetterhoff:** None. **A.J. Sweatt:** None. **S.A. Deadwyler:** None. **R.E. Hampson:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.06/JJJ49

Topic: F.02. Animal Cognition and Behavior

Support: DSP Support

Title: Relationship of cortical dopamine, acetylcholine, serotonin, glutamate, and GABA to restoration of novel object recognition by antipsychotic and specific receptor agonists or antagonists

Authors: *M. HUANG[1], J. J. PANOS[1], S. KWON[1], Y. OYAMADA[1,2], L. RAJAGOPAL[1], H. Y. MELTZER[1];
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Abstract: Background: The ability of atypical antipsychotic drugs (APDs) to improve some domains of cognitive impairment of schizophrenia may be due, in part, to increased release of dopamine (DA) and acetylcholine (ACh) in various cortical and temporal lobe regions. It has been reported that the cortical or hippocampal DA, ACh, and glutamate (Glu) were increased during the cognitive processes related to attention and working memory. Sub-chronic(sc) phencyclidine (PCP), an NMDA receptor non-competitive antagonist, which induces impairment in glutamatergic neurotransmission impairs novel object recognition (NOR) in rodents and primates. Our previous studies have shown this deficit can be acutely reversed by administration of atypical, but not typical APDs, by 5-HT_{1A} partial agonists (PA), but not 5-HT_{2A} inverse agonists (IA). **Method:** In the present study, using microdialysis and UPLC-MS/MS to assay neurotransmitter efflux in awake freely moving rats, we compared the effect of the atypical APDs blonanserin (D_2/5-HT_{2A} receptor antagonist, 3 mg/kg, ip), lurasidone (5-HT_7/5-HT_{2A}/D_2 receptor antagonist, 5-HT_{1A} PA, 0.5 mg/kg), and olanzapine (5-HT_{2A}/D_2/5-HT_6 receptor antagonist, 1 mg/kg), as well as the typical APD, D_2 receptor antagonist, haloperidol (0.1 mg/kg), the 5-HT_{1A} PA tandospirone (5 mg/kg), and pimavanserin (3 mg/kg), a 5-HT_{2A} IA, on the efflux of cortical DA, ACh, Glu, GABA, serine, glycine, 5-HT, and the metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillac acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA). **Results:** All compounds, with the exception of haloperidol and pimavanserin, increased the efflux of cortical DA and its metabolites, DOPAC and HVA. Only olanzapine and lurasidone increased cortical ACh efflux. Only lurasidone slightly, but significantly, increased cortical Glu efflux. None of the compounds, at the doses tested, significantly altered the efflux of cortical 5-HT or its metabolite, or glycine or GABA. **Conclusion and Discussion:** The present data suggests that atypical APDs, probably due to 5-HT_{2A} receptor blockade, combined with lesser D_2 receptor blockade, and direct or indirect5-HT_{1A} receptor activation, but not D_2 or 5-HT_{2A} receptor blockade alone, increases cortical DA and, in some cases, ACh efflux. The Glu efflux induced by lurasidone could possibly be related to its potent 5-HT_7 receptor blockade. The ability to increase cortical DA, ACh as well as Glu efflux might contribute to amelioration of the deficit in NOR following scPCP treatment. Additional data testing this conclusion and its relation to NOR in scPCP treated rodents will be reported at the SFN meeting.

Disclosures: M. Huang: None. **J.J. Panos:** None. **S. Kwon:** None. **Y. Oyamada:** None. **L. Rajagopal:** None. **H.Y. Meltzer:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.07/JJJ50

Topic: F.02. Animal Cognition and Behavior

Title: Estradiol enhances object memory through estrogen receptor α, but not β, activation in the hippocampus of female mice

Authors: *L. M. PEREIRA, C. P. BASTOS, G. S. PEREIRA;
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Abstract: 17β-Estradiol (E2) enhances hippocampal function and improves performance in several memory tasks. However, the molecular mechanisms underlying estrogenic-induced promnesic effects remain unclear. In the present study, we asked whether E2 exert its mnemonics effects through the estrogen receptors alfa (ERα) and beta (ERβ). To assess the object recognition memory we used the Novel Object Recognition task. First, we demonstrate that in Swiss ovariectomized mice post-training intraperitoneal (i.p.) injection of E2 (0,2mgKg) enhances the retention of object recognition memory. Second, we show that this effect is reproduced by intrahippocampal administration of PPT (1μg/μl), a selective ERα agonist, but not of DPN (0,4 and 2μg/μl), a selective ERβ agonist. We confirm this result blocking the mnemonic effect of i.p. E2 by dorsal hippocampal inhibition of ERα activation. Animals received i.p. injection of E2 followed by intrahippocampal administration of TPBM (10, 50 and 250ng/μl), a selective ERα antagonist. We also demonstrate that DPN, but not PPT, intrahippocampal administration has anxiolytic-like effect, in the elevated plus-maze, in Swiss mice and that none of these drugs has effects in the locomotor activity. Taken together, these data suggest that E2 acts as a cognitive enhancer through activation of dorsal hippocampal ERα.

Disclosures: L.M. Pereira: None. **C.P. Bastos:** None. **G.S. Pereira:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

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Program#/Poster#: 287.08/JJJ51

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant DA07625

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DARPA N66001-09-C-2080

NIH training grant 5T32-NS007422

Title: Role of cannabinoid system in hippocampal memory

Authors: D. FETTERHOFF, *A. J. SWEATT, W. D. JOHNSTON, C. A. SEXTON, R. E. HAMPSON, S. A. DEADWYLER;
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Abstract: Activation of the brain cannabinoid (CB1) receptor produces a clear modulation of memory in rats performing a Delayed-Nonmatch-to-Sample (DNMS) task. Exogenously applied CB1R agonists reduced behavioral performance and suppressed the hippocampal neural response which encodes task-critical information [Hampson & Deadwyler, J. Neurosci. 2000; 20(23):8932-42]. On the other hand, CB1R antagonists enhance behavioral performance and hippocampal neural activity in some but not all behavioral contexts [Deadwyler et al. Behav. Pharmacol. 2007; 18(5-6):571-80]. The reciprocal nature of CB1R activation/inactivation suggests that endocannabinoid modulate memory processes as part of the normal function of the hippocampus; however, demonstrating such a role via direct manipulation of endocannabinoids has proven to be much more difficult.

This study examined the role of cannabinoid signaling in memory by manipulating endocannabinoid signaling with CB1R activation/inactivation and direct electrical stimulation of hippocampal neural ensembles. Inhibition of endocannabinoid degradation by inhibition of fatty-acid-amide-hydrolase via URB597, or by inhibition of MAG-lipase via URB602, produced mnemonic impairment in two ways: a) DNMS performance was impaired at long delays by both URB597 and URB602, and b) hippocampal neuronal encoding of Sample phase information in the DNMS task was suppressed by both URB597 and URB602. The CB1R antagonist rimonabant blocked behavioral impairment produced by exogenous or endogenous cannabinoids, and also strengthened hippocampal neural encoding of task-relevant information in the DNMS task. Patterned electrical stimulation of hippocampal neurons reversed CB1R agonist-induced behavioral impairments, implying that CB1Rs specifically modulate the encoding of mnemonic information critical to task performance. To further understand the neural correlates associated with manipulating the endocannabinoid system, the neural recordings were used to estimate the multifractal singularity spectrum using the Wavelet Leaders method (Serrano and Figliola, Physica A, 2009; 388:2793-2805; Wendt et al., IEEE Signal Processing, 2007; 24:38-48). Preliminary results show that changes in the multifractal spectrum due to CB1R activation or inactivation are directly related to the functional role of the neuron during the DNMS task. Together these results suggest that the differential modulation of CB1Rs via the action of endocannabinoids may play a normal role in memory processing, and may enhance future understanding of cannabinoid signaling with respect to information processing by the brain.

Disclosures: D. Fetterhoff: None. **W.D. Johnston:** None. **A.J. Sweatt:** None. **C.A. Sexton:** None. **R.E. Hampson:** None. **S.A. Deadwyler:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.09/JJJ52

Topic: F.02. Animal Cognition and Behavior

Title: Improved neuritogenesis and mitochondrial dynamics by levetiracetam might explain cognitive improvement in brain aging and animal models of Alzheimer's disease

Authors: *W. E. MUELLER[1], D. VIANO[2], C. SCHILLER[2], K. LEUNER[3];
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Abstract: The antiepileptic levetiracetam (Lev) has been shown to improve hippocampal hyperactivity associated with Mild Cognitive Impairment (MCI) in patients and cognitive deficits in an animal model of Alzheimer's disease (AD). These effects have been explained by improvement of synaptic function but the mechanism has not yet been clarified, also effects on mitochondrial function seem to be involved. Since loss of synapses and neurites associated with impaired mitochondrial function and dynamics (fission and fusion) are typical for brain aging and early AD, we assessed the effects of Lev on neurite outgrowth and mitochondrial parameters. Human neuroblastoma cells (SY5Y) and PC12 cells were used under conditions imitating aging and SY5Y cells expressing slightly higher beta-amyloid levels typical for very early stages of AD. These cells exhibit impaired neuritogenesis and mitochondrial dynamics already under baseline conditions and/or after treatment with rotenone. Lev at a concentration as low as 20 micromolar improves neuritogenesis and mitochondrial dynamics in both cell lines following oxidative stress either induced by rotenone treatment (complex I inhibition) or by sodium nitroprusside (SNP). At similar concentrations levetiracetam also had beneficial effects on both parameters in SY5Y cells overexpressing beta-amyloid. Improved neuroplasticity followed by improved neuronal communication might be associated with the beneficial effects of Lev on cognitive functions in patients and Alzheimer mice independent of its anticonvulsant properties.

Disclosures: W.E. Mueller: 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.; UCB, Schwabe. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); UCB,Schwabe,Lundbeck. F. Consulting Fees (e.g., advisory boards); UCB,Schwabe,Lundbeck. **D. Viano:** None. **C. Schiller:** None. **K. Leuner:** 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.; UCB, Schwabe. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents' (e.g., speakers' bureaus); UCB.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.10/JJJ53

Topic: F.02. Animal Cognition and Behavior

Support: DSP Support

Title: TPA023, a GABA($_A$) α2,3 subtype-selective partial agonist, enhances the efficacy of lurasidone to reverse the sub-chronic phencyclidine (PCP)-induced impairment in novel object recognition (NOR)

Authors: *Y. OYAMADA[1,2], L. RAJAGOPAL[1], M. HUANG[1], H. Y. MELTZER[1];
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Abstract: Background: Cognitive impairment in schizophrenia (CIS), reflects deficits of multiple cognitive domains, among them being declarative memory (DM), primarily a hippocampal-based function. CIS is believed to result from hypoglutamate, deficient $GABA_A$ receptor function, and diminished dopamine D_1 receptor stimulation. Typical and atypical antipsychotic drugs (APDs) ameliorate deficits in DM in some patients beyond a practice effect (Meltzer et al., 1989; Keefe et al., 2007). We have previously reported the effectiveness of the novel antipsychotic drug, lurasidone (a D_2 antagonist, 5-HT_{1A} partial agonist, and 5-HT_{2A} and 5-HT_7 antagonist) and the selective 5-HT_{1A} receptor agonist, tandospirone, in reversing the deficit in novel object recognition (NOR), an analog of DM, following sub-chronic (sc) treatment with the NMDA receptor non-competitive antagonist, PCP, in rats (Horiguchi et al., 2011). The use of add-on pharmacological agents for CIS might enhance the efficacy of atypical APDs such as lurasidone. In this light, TPA023, a GABA type A receptor subunit α2/α3 positive allosteric

modulator, was studied as adjunctive for lurasidone and tandospirone in rat NOR. We also studied the effect of the $GABA_A$ antagonist, bicuculline alone or in combination with TPA023 on NOR. **Materials and Methods:** Female Long-Evans rats received vehicle or PCP (2 mg/kg, b.i.d.) for 7 days, followed by a 7-day washout. PCP-treated rats were given TPA023 (0.01 or 0.05 mg/kg) or bicuculline (0.1 or 0.5 mg/kg), alone, or along with a sub-effective dose (SED) of lurasidone (0.03 mg/kg), or tandospirone (0.2 mg/kg), 30 min prior to NOR testing. The NOR procedure has been described elsewhere (Snigdha et al., 2010). **Results:** Acute treatment with TPA023 (0.05 mg/kg), but not 0.01 mg/kg, significantly reversed the scPCP-induced NOR deficit. Acute treatment with, the $GABA_A$ antagonist, bicuculline (0.5 mg/kg), but not 0.1 mg/kg, also significantly reversed the scPCP-induced NOR deficit. Co-administration of SED of TPA023 (0.01 mg/kg) with SED of lurasidone (0.03 mg/kg), but not tandospirone (0.2 mg/kg), enhanced the ability of lurasidone to reverse this deficit. Additional data with bicuculline pretreatment of lurasidone and tandospirone will be reported.
Conclusion and Discussion: These findings demonstrate the significance of the $GABA_A$ mechanism for the cognitive deficits induced by scPCP, a putative model for CIS, alone and as adjunctive treatment with lurasidone and possible other APDs. The amelioration of NOR deficit by both agonist and antagonist could be attributed to their effect on the number of $GABA_A$ receptors.

Disclosures: Y. Oyamada: A. Employment/Salary (full or part-time):; DSP employee. **L. Rajagopal:** None. **M. Huang:** None. **H.Y. Meltzer:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.11/JJJ54

Topic: F.02. Animal Cognition and Behavior

Support: The National Natural Science Funds (NO. 81171031)

Title: Involvement of GSK3β/β-catenin signaling in the impairment effect of ketamine on memory

Authors: *X. LIU[1], H. LIU[1], K. WANG[2], G. XU[1], J. CAO[3], Y. LI[1];
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Abstract: Background: Ketamine produces acceptable analgesia, it is accompanied by amnesia. The cellular mechanisms underlying ketamine induced amnesia are not clear. The current

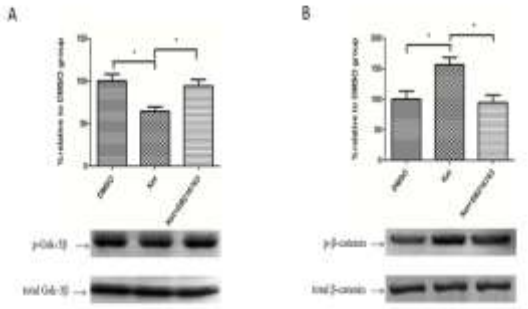
experiments were designed to examine the effects of ketamine on memory consolidation in rats, and, more precisely, whether the glycogen synthase kinase (GSK)3β/β-catenin signaling pathway was involved in mediating the effects of posttraining ketamine administration on Morris water maze retention.

Methods: Adult male Sprague-Dawley rats were injected with ketamine (25, 50,100 mg/kg) immediately after a water maze training session consisting of eight trials. A probe trial was carried out 24 h later to examine the effects of ketamine on memory. The rats' hippocampi were obtained for measurement of levels of different forms of GSK3β and β-catenin protein expression.

Results: Memory performance was significantly impaired in rats injected with ketamine (100 mg/kg) after training. This dose altered activation of GSK3β/β-catenin signaling pathway in the hippocampus. Acute injection of the GSK3β specific inhibitor SB216763 (1 ng/0.5 μl/side) into area CA1 of the hippocampus after water maze training prevented ketamine-induced impairment of memory consolidation and blocked ketamine-induced effects on the GSK3β/β-catenin signaling pathway in the hippocampus.

Conclusion: An anesthetic dose of ketamine injected immediately after Morris water maze training impaired memory consolidation. Pharmacological inhibition GSK3β/β-catenin signaling blocked the memory impairing effect of ketamine, suggesting that GSK3β/β-catenin signaling may play a role in ketamine-induced amnesia.

*Fig.6. Administration of SB216763 immediately after water maze training blocks the effects of 100 mg/kg ketamine on the phosphorylation state of GSK-3β (A) and β-catenin (B) in the hippocampus. Data are shown as mean percentage levels (±SEM) with respect to the DMSO vehicle control group.*p < 0.05, Tukey's test after one-way ANOVA, n = 5 each group.*



Disclosures: X. Liu: None. **H. Liu:** None. **K. Wang:** None. **G. xu:** None. **J. Cao:** None. **Y. Li:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.12/JJJ55

Topic: F.02. Animal Cognition and Behavior

Title: Oleanolic acid attenuates scopolamine-induced memory impairment in mice

Authors: Y. LEE[1], S. PARK[1], H. LEE[1], Y. AHN[1], G. KWON[1], H. WOO[1], Q. GAO[1], *J. RYU[2,1];
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Abstract: Oleanolic acid and ursolic acid are pentacyclic triterpenoids that are found in medicinal herbs as well as foods. In the present study, we investigated the memory-ameliorating effect of these two triterpenoids on cholinergic blockade-induced memory impairment in mice. The behavioral tests were conducted to evaluate the memory-ameliorating effect of both oleanolic acid and ursolic acid. Cognitive impairment was induced by scopolamine (1 mg/kg), a muscarinic receptor blocker, in prior to 30 min before the behavioral tests. Oleanolic acid (2.5 or 5 mg/kg, p.o.) significantly ameliorated the scopolamine-induced cognitive impairment, whereas ursolic acid did not exert any memory-ameliorating effect in our present behavioral tasks. To clarify the mechanism underlying memory-ameliorating effect of oleanolic acid, the receptor antagonism study and Western blot analysis were conducted. We will discuss on the structural differences between oleanolic acid and ursolic acid which are responsible for cognitive function.

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Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.13/JJJ56

Topic: F.02. Animal Cognition and Behavior

Support: Institute for Basic Science

Title: Distinct cortical connectivity pattern of the anterior and posterior mediodorsal thalamic nucleus in mice

Authors: *J. LEE[1], F. MÁTYÁS[2], L. ACSÁDY[2], H.-S. SHIN[1];
[1]Ctr. for Cognition and Sociality, Inst. For Basic Sci., Seoul, Korea, Republic of; [2]Inst. of Exptl. Med. of the Hungarian Acad. of Sci., Budapest, Hungary

Abstract: The mediodorsal thalamic nuclei (MD) provide one of the most significant thalamic inputs to the frontal cortices and plays pivotal role in frontal cortical function including attention, planning and decision-making. Indeed, MD lesions results in similar behavioral symptoms than the lesion of the cortical region they are connected with, which underlies the importance to understand the precise connectivity between the two regions. Earlier investigators described the topographic connection between the medial thalamus and frontal cortex as serial, parallel loops via the basal ganglia in rats. In this study we used anterograde and rertrograde tracing to study the connectivity of MD in various antero-posterior levels in mice. The study was motivated by our recent study which that the MD also has a vital role in fear extinction, a classical prefrontal function.
Injection of the retrograde tracers to the caudal MD labeled cortical layer 6 and layer 5 cells as described previously. The vast majority of the labeled cell was in the primary motor cortex. In addition, significant amount of labeled cell was found in the secondary motor, dorsal cingulate, primary somatosensory, agranular insular and perirhinal cortices but not in the pre- or infralimbic cortices. More anterior injections, however, resulted in dense labeling of the deep layers of pre-, infralimbic and agranular insular cortices but not in the somato-motor cortices. A moderate number of contralateral corticothalamic cells were also observed in both cases. Double-labeling experiments suggested an abrupt rather than a gradual change in the cortical innervation pattern of rostral vs. caudal MD. Distinct cortical inputs were paralleled by distinct thalamic reticular nucleus (TRN) inputs arising from different sectors of the nucleus. Indeed, only few TRN cells were found which projected to both the anterior and posterior MD. Interestingly retrogradely labeled cells were not found in the amygdala following either anterior or posterior MD injections, which was confirmed by the lack of fibers in the MD after anterograde tracing from both the basolateral and the central amygdalar nuclei.
Based on the cortical innervation pattern, our results suggest a functional heterogeneity in the MD. The data suggest the caudal MD is involved in motor execution and planning, whereas rostral MD is responsible for emotional behavior, like fear and anxiety.

Disclosures: J. Lee: None. **F. Mátyás:** None. **L. Acsády:** None. **H. Shin:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.14/JJJ57

Topic: F.02. Animal Cognition and Behavior

Support: CSIR, New Delhi

Title: Naringin ameliorates okadaic acid induced cognitive dysfunction and apoptotic neurodegeneration in the rat brain

Authors: *A. K. SACHDEVA, K. CHOPRA;
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Abstract: Tau hyperphosphorylation and memory deficit are characteristic alterations of Alzheimer's disease (AD). Protein phosphatases (PP) 2A plays a crucial role in AD-like lesions. Inhibition of PP2A through intracerebroventricular injection of okadaic acid (ICV-OKA) induces tau hyperphosphorylation and memory impairment. In the present study, we investigated the effect of naringin on ICV-OKA-induced cognitive impairment in rats. ICV-OKA administration produced significant cognitive deficits as assessed by both Morris water maze and elevated plus maze task which is accompanied with significantly enhanced oxidative-nitrosative stress, acetylcholinesterase, and mitochondrial enzyme (I, II,III and IV) activities and surge in serum inflammatory cytokines (TNF-α, TGF-β and IL-1β. Chronic treatment with naringin, (50, 100 and 200 mg/kg; oral gavage) for 14 days significantly and dose dependently improved cognitive deficits in ICV-OKA rats along with mitigation of oxido-nitrosative stress mediated alterations in mitochondrial enzyme activities and serum inflammatory cytokines. Naringin produced comparable effects to rivastigmine (2mg/kg; po). Thus the study demonstrates the effectiveness of naringin in preventing cognitive deficits caused by ICV-OKA in rats and it may provide a novel and effective strategy to treat neurodegenerative diseases such as Alzheimer's disease.

Disclosures: A.K. Sachdeva: None. **K. Chopra:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.15/JJJ58

Topic: F.02. Animal Cognition and Behavior

Support: Institute for Basic Science

Title: The precision of contextual familiarity memory is attenuated in remote retrieval

Authors: *J. PARK, H. SHIN;
Ctr. for Cognition and Sociality, IBS, Seoul, Korea, Republic of

Abstract: The research to elucidate the neural basis of remote memory has been mostly studied only within the small fields, such as fear conditioning. Our daily life is, however, usually in neutral contexts without severely fearful incidents. It has been known that a decrease of locomotive behavior is frequently observed in rodents when exposed to a familiar circumstance. Thus, the memory level for a given context can be measured at this moment. This methodology was employed to investigate the characteristics of remote memory in neutral circumstance. We recruited 2 kinds of sensory modalities, box shape and odor, which of two are useful to distinguish open filed contexts. The context "A" consists of square shape and cineole odor while the context "B" is composed of circular shape and heptanole odor. These two types of conditions were used either for a novel or a familiar contextual environment. In order to induce distinct types of contextual familiarity memory, one group of mice was exposed to the context A, and the other, the context B. The retrieval of remote memory was tested 6 weeks following each initial exposure. To evaluate whether locomotive behavior was actually reduced in the only experienced context, mice were exposed to novel context again 1 day after the remote memory test. Interestingly, exposures to both contexts were resulted in decreased locomotive behavior in the mouse. It was confirmed that mice could distinguish two different contexts in recent retrieval, and even 3 consecutive days of training could not prevent the attenuation of memory precision in remote retrieval. Here I report that contextual familiarity memory is generalized in remote, but not recent retrieval.

Disclosures: J. Park: None. **H. Shin:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.16/JJJ59

Topic: F.02. Animal Cognition and Behavior

Support: NIMH grant R01MH084038

NIMH grant R21MH082417

Title: Mechanism of the schizophrenia-related cognitive control impairment induced by phencyclidine

Authors: *H.-Y. KAO[1], E. PARK[1], B. RADWAN[1], M. T. VAN DIJK[2], E. WALLACE[3], M. J. TROY-REGIER[3], J. ZHONG[1], J. M. ALARCON[4], A. A. FENTON[1,5];
[1]Ctr. for Neural Sci., [2]Sackler Inst. of Grad. Biomed. Sci., New York Univ., New York, NY; [3]Sch. of Grad. Studies, Program of Neural and Behavioral Sci., [4]Dept. of Pathology, [5]Dept. of Physiol. and Pharmacol., State Univ. of New York at Downstate Med. Ctr., Brooklyn, NY

Abstract: Impaired cognitive control, an inability to use relevant information and ignore salient but irrelevant information, is perhaps the most debilitating deficit in schizophrenia but this and other cognitive symptoms are not treated by antipsychotic medications. The development of procognitive antipsychotics is stymied by the lack of mechanistic hypotheses both for the cognitive deficits and model systems for evaluating them. We examined the "discoordination hypothesis" that discoordination of electrical neural activity underlies cognitive dysfunction. We show, in the most widely used animal model for developing antipsychotics, that the psychotomimetic phencyclidine (PCP) impaired cognitive control in rodents performing a two-frame active place avoidance task that requires using relevant cues and ignoring irrelevant cues to avoid a shock zone. PCP also discoordinated the electrical activity in networks of hippocampal CA1 place cells at the levels of both ensemble action potential discharge and field potential oscillations, without disrupting the spatial firing of individual place cells.
We then sought the molecular mechanism of this PCP-induced neural and cognitive discoordination. Multiple findings indicate that PCP rapidly induces aberrant group 1 mGluR stimulated protein synthesis. We injected mice with PCP or vehicle, and hippocampal slices were obtained to assess the protein synthesis dependent mGluR-stimulated LTD at the Schaffer collaterals to CA1 synapses by bath application of the group I mGluR agonist DHPG. Anisomycin, a protein synthesis inhibitor, given prior to the PCP injection, blocked the PCP-induced increase of mGluR-LTD, prevented the PCP-induced impairment of cognitive control as well as discoordination of neural spike trains. The PCP-induced cognitive control impairment was also prevented by pretreating mice with MPEP, the group I mGluR antagonist.
These findings indicate that PCP causes rapid group 1 mGluR-stimulated protein synthesis that leads to neural discoordination and cognitive control failures. These data i) strongly support the hypothesis that neural discoordination is a central pathophysiology underlying cognitive dysfunction in schizophrenia, ii) identify a novel biochemical cause of the pathophysiology, and iii) provide both a rationale and experimental platform for developing procognitive antipsychotic medications.

Disclosures: H. Kao: None. **E. Park:** None. **B. Radwan:** None. **M.T. van Dijk:** None. **E. Wallace:** None. **M.J. Troy-Regier:** None. **J. Zhong:** None. **J.M. Alarcon:** None. **A.A. Fenton:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.17/JJJ60

Topic: F.02. Animal Cognition and Behavior

Support: Institute for Basic Science

Title: Activity of extrasynaptic gabaa receptors in mediodorsal nucleus of thalamus modulates fear extinction learning

Authors: *A. PAYDAR, S. LEE, B. LEE, G. GANGADHARAN, H.-S. SHIN;
Ctr. for Cognition and Sociality, Inst. For Basic Sci., Seoul, Korea, Republic of

Abstract: In fear extinction repetitive exposure of a conditioned animal to conditional stimulus (CS) alone will result in decrease of conditional response (CR). This approach can be used for treating patients with anxiety disorders, e.g. phobias and post-traumatic stress disorder (PTSD). Prefrontal cortex (PFC), amygdala and hippocampus are essential for fear extinction. Mediodorsal nucleus (MD) of thalamus has been implicated in fear extinction learning. GABA, the main inhibitory neurotransmitter in the brain, has an essential role in the fear extinction. GABAA receptors provide phasic (through synaptic GABAA receptors) and tonic (via extrasynaptic GABAA receptors) inhibition.
In this study we investigated the role of GABAergic activity in MD in fear extinction learning. Injection of Gabazine, a GABAA receptor antagonist, into MD region of mice brain before fear extinction decreased freezing behavior in response to tone (CS), i.e. facilitated fear extinction learning. Also, injection of Gaboxadol (THIP), an extrasynaptic GABAA receptor agonist, into the MD region before extinction, attenuated fear extinction.
In addition to confirming the role of MD in fear extinction learning, our results show the GABAergic activity in MD nucleus of Thalamus, especially the “tonic GABA inhibition” resulted from the extrasynaptic GABAA receptors, modulates the fear extinction learning.

Disclosures: A. Paydar: None. **S. Lee:** None. **G. Gangadharan:** None. **H. Shin:** None. **B. Lee:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.18/JJJ61

Topic: F.02. Animal Cognition and Behavior

Support: Institute for Basic Science

Title: Cav3.1 T-type Ca2+ channels controls hippocampal theta oscillations and novelty behavior in mice

Authors: *G. GANGADHARAN[1], J. SHIN[2], S.-W. KIM[1], A. KIM[1], D.-S. KIM[3], A. PAYDAR[1], M. WATANABE[4], Y. YANAGAWA[5], Y.-S. KIM[6], D. KIM[7], H.-S. SHIN[1]; [1]Ctr. for Cognition and Sociality, Inst. for Basic Sci. (IBS), Seoul, Korea, Republic of; [2]Dept. of Psychiatry and Biobehavioral Sci., UCLA, CA; [3]Dept. of Anat., Col. of Medicine, Soonchunhyang Univ., Cheonan-Si, Korea, Republic of; [4]Dept. of Anat., Hokkaido Univ. Sch. of Med., Sapporo, Japan; [5]Dept. of Genet. and Behavioral Neurosci., Gunma Univ. Grad. Sch. of Med., Maebashi, Japan; [6]Dept. of Smart Foods and Drugs and Indang Inst. of Mol. Biol., Inje Univ., Seoul, Korea, Republic of; [7]Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Hippocampal theta oscillations are implicated in diverse brain functions including novelty behavior. Although T-type calcium channels are crucial for the genesis of many neuronal oscillations and different behaviors, it remains unexamined whether these channels control theta oscillations and associated behaviors. Here we show that mice with a knockout or medial septal-specific knockdown of Cav3.1 T- type channels exhibited increased cholinergic theta oscillations and enhanced novelty behavior. Cav3.1 channels are strongly expressed in GABAergic neurons in the medial septum. Interestingly, in the mutant mice the septo-hippocampal projecting GABAergic neurons lacked low threshold spikes and markedly increased spontaneous discharge activities. Moreover, optogenetic stimulation of septo-hippocampal GABAergic neurons reproduced the increased novelty behavior shown by the Cav3.1 knockout or knockdown mice. These results highlight the new role of Cav3.1 T-type calcium channels in the control of hippocampal cholinergic theta oscillations and novelty behavior.

Disclosures: G. Gangadharan: None. **J. Shin:** None. **S. Kim:** None. **A. Kim:** None. **D. Kim:** None. **A. Paydar:** None. **M. Watanabe:** None. **Y. Yanagawa:** None. **Y. Kim:** None. **D. Kim:** None. **H. Shin:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.19/JJJ62

Topic: F.02. Animal Cognition and Behavior

Title: Drug injectable carbon-nanotube nanoelectrodes

Authors: *J. SHIN[1,2], G. KIM[1], I. KIM[1], T. AN[4], H.-S. SHIN[3,5], G. LIM[6,7];
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Abstract: Microelectrodes are widely used for monitoring neural activities in various neurobiological studies, but these devices either can damage cells during penetration or are incapable of drug injection. Here, we report carbon-nanotube-based nanoelectrodes with a diameter of ~300nm for interrogating cells, transporting fluids at the single cell level. The carbon-nanotube wire was fabricated using dielectrophoresis and surface tension which is much more easy-to-make and shape-controllable technique than previously a carbon-nanotube pipette (CNP). For neural electrodes, the nanoelectrodes have excellent electrochemical properties such as low impedance, large electrochemical surface area and ohmic contact characteristic. In addition, the nanoelectrode, which is made by placing a single-walled nanotube at the tip of a glass pipette, can probe the intracellular environment and deliver specific drug to the target cell without disrupting the cell. Because they are combined on conventional micropipettes, the nanoelectrodes are readily applied to traditional instruments, creating various opportunities for minimally invasive intracellular recording, and drug delivery.
Keywords: carbon-nanotubes (CNTs), nanoelectrode, glass pipette, neural recording, drug injection

Disclosures: J. Shin: None. **G. Kim:** None. **I. Kim:** None. **T. An:** None. **H. Shin:** None. **G. Lim:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.20/JJJ63

Topic: F.02. Animal Cognition and Behavior

Title: CE-245677-induced cognitive deficits: A translational story implicating inhibition of trkb-mediated pathways

Authors: *A. N. FOOTE[1], C. HSU[1], A. MEAD[1], R. NEWMAN[2], K. CANNON[1];
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Abstract: CE-245677 is an antagonist at Tie2 (IC_{50} = 0.28 nM), a member of the receptor tyrosine kinase family, and was in Phase 1 clinical trials for the treatment of solid tumor growth. Administration of 100 mg CE-245677 q.i.d. resulted in a spectrum of cognitive dysfunction that ranged from mental fogginess appearing as early as day 2 to memory loss that appeared by day 15 in 3 out of 4 patients (IND Annual Report, CE-245677). Total plasma exposure of CE-245677 from the patient presenting with mental fogginess on day 2 was approximately 814 ng/mL. Nonclinically, the contextual renewal (CR) assay was used to assess alterations in cognitive performance after acute oral administration of CE-245677 to male Wistar Han rats. The CR model assesses the ability of a rat to learn simple associations between environmental cues and rewarding stimuli (i.e., food). When administered as a positive control, 1 mg/kg s.c. scopolamine decreased memory recall and cue-induced renewal responding in the CR assay. Oral administration of 0.3 mg/kg CE-245677 failed to produce changes in memory recall or cue-induced renewal responding in this assay. However, oral administration of 10 or 100 mg/kg CE-245677 decreased memory recall and cue-induced renewal responding when compared to the renewal vehicle, thus suggesting that these doses of CE-245677 alter aspects of cognitive performance in rats. The total plasma exposure levels of CE-245677 were 46.5, 802, and 7458 ng/mL following administration of 0.3, 10, and 100 mg/kg, respectively. Calculated from total brain exposures levels (ppb = 0.99), the estimated free brain exposure levels of CE-245677 were 0.5, 9, and 73 nM following administration of 0.3, 10, and 100 mg/kg.
In addition to activity at Tie2, CE-245677 also possess inhibitory secondary pharmacology at TrkB receptors (TrkB K_i = 4.8 nM). TrkB receptors have been hypothesized to play a role in learning and memory processes (Yamada & Nabeshima, 2003). Thus, inhibition of these receptors may lead to cognitive impairment. Given the putative role of TrkB receptors in cognitive processes, it is possible that CE-245677-induced alterations in cognitive performance observed both clinically and nonclinically may be a result of off-target activity at TrkB receptors. In support of this, estimated free brain concentrations of 10 and 100 mg/kg CE-245677 in rats were approximately 1.9x and 15.1x, respectively, over CE-245677's K_i for TrkB. From the clinical data, the estimated free brain concentration was approximately 2x over the compound's K_i for TrkB. These findings suggest that CE-245677-induced alterations in cognitive performance may be a result of TrkB inhibition in the brain.

Disclosures: A.N. Foote: None. **C. Hsu:** None. **A. Mead:** None. **R. Newman:** None. **K. Cannon:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.21/JJJ64

Topic: F.02. Animal Cognition and Behavior

Support: NSERC Discovery Grant 400176

Title: The role of striatal cholinergic transmission in cognition: Assessment of mice deficient for the vesicular acetylcholine transporter (VAChT) in the striatum

Authors: *D. R. PALMER[1], K. GAHNSMITH[2], R. COURTNEY[2], I. PLUMB[2], B. BULOSAN[2], E. BINSELL[2], A. VAN MAANEN[2], S. CREIGHTON[2], V. F. PRADO[3], M. M. PRADO[3], E. CHOLERIS[1], B. D. WINTERS[1];
[1]Psychology: Neurosci. and Applied Cognitive Sci., [2]Univ. of Guelph, Guelph, ON, Canada; [3]Dept. of Physiol. & Pharmacol., Western Univ., London, ON, Canada

Abstract: The neurotransmitter Acetylcholine (ACh) has been implicated in numerous aspects of cognition including learning, memory, and attention. Previous research has indicated important roles for the cholinergic neurons of the basal forebrain in many of these functions. In addition to the basal forebrain, however, there are other cholinergic networks in the brain that may influence cognition. For example, the striatum contains a population of cholinergic interneurons that help to regulate basal ganglia activity. At present, it is not clear whether these cholinergic interneurons play a role in cognition. Recently, a strain of genetically modified mice have been developed which are deficient for the Vesicular Acetylcholine Transporter (VAChT) within the striatum (Guzman et al, 2011). We tested these Striatal VAChT mice on a battery of learning, memory, attention, and cognitive flexibility tasks. Striatal VAChT mice were impaired on a spontaneous object recognition task when the delay between initial learning and memory testing was 15-minutes, but not when the delay was extended to 3 hours. Impairment was also observed in male VAChT mice tested on a social recognition paradigm with the short delay, but not the long delay. Similar impairment was not seen on a spontaneous object location task assessing spatial memory. On a mouse touchscreen 5-choice serial reaction time task it was found that striatal VAChT mice showed impairments in attention, as accuracy was reduced and omission rates were increased. To assess associative learning and cognitive flexibility, mice were also trained on a visual pairwise discrimination task using touchscreens. Striatal VAChT mice acquired the visual discrimination normally; interestingly, however, they were impaired on subsequent trials where the reward contingency was reversed, suggesting a deficit in cognitive flexibility. In all, these results indicate that striatal cholinergic transmission is involved in short-term social and object recognition processing, attentional processing, and the reversal of highly

trained associations. These effects may be related to striatal cholinergic influences on interactions between the dorsal striatum and prefrontal cortex.

Disclosures: D.R. Palmer: None. **K. Gahnsmith:** None. **R. Courtney:** None. **I. Plumb:** None. **B. Bulosan:** None. **E. Binsell:** None. **A. Van Maanen:** None. **S. Creighton:** None. **V.F. Prado:** None. **M.M. Prado:** None. **E. Choleris:** None. **B.D. Winters:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.22/JJJ65

Topic: F.02. Animal Cognition and Behavior

Support: FIRST (SI)

KAKENHI 22241048 (SI)

RIKEN Junior Research Associate Program (KY)

Title: Genetic analysis of the roles of NMDA receptors in the parafascicular nucleus in sensory processing

Authors: *K. YASUDA[1,2], Y. HAYASHI[1], M. TANAKA[1], S. ITOHARA[1];
[1]RIKEN Brain Sci. Inst., Wako-Shi, Saitama, Japan; [2]Grad. Sch. of Agr. and Life Sci., 1-1-1, Yayoi, Bunkyo-ku, Tokyo, Japan

Abstract: Distinct brain regions perform different types of computations for processing sensory information to support various behaviors. Little is known, however, about how sensory information processed in individual brain areas is integrated.
The parafascicular nucleus (Pfn), an intralaminar thalamic nucleus, might have a special role in integrating sensory information. The Pfn has abundant mutual connections with two specific brain systems: the anterior cingulate cortex and the striatum. The anterior cingulate cortex and striatum are involved in the selection of sensory stimuli and behavioral output, respectively. Therefore, the Pfn might serve an important role in selecting sensory inputs that are relevant to behavioral decision. Neurons of the Pf are activated by unfamiliar or unexpected stimuli, and these firing responses often habituate rapidly when the same stimuli are presented repeatedly. We postulate that the habituation serves as a mechanism for selecting salient or task-relevant stimuli, and that the rapid habituation of Pf neurons is modulated by NMDA receptors.
To test this hypothesis, we generated Pfn-selective conditional knockout (cKO) mice of NR1,

which encodes an essential NMDA receptor subunit. We developed a transgenic mouse line expressing Cre selectively in this brain area and crossed these mice with mice in which NR1 is flanked by loxP. These cKO mice exhibited hyperactivity in their home cages, although there was no evidence of hyperactivity in a novel environment. Furthermore, they displayed motor coordination deficits on the accelerating rotarod. These preliminary results support the notion that the rapid habituation of Pfn neurons via NMDA receptors is crucial for the selection of task-relevant sensory information.

Disclosures: K. Yasuda: None. **Y. Hayashi:** None. **M. Tanaka:** None. **S. Itohara:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.23/JJJ66

Topic: F.02. Animal Cognition and Behavior

Title: Microinfusions of estradiol to the medial prefrontal cortex bias adult female rats towards use of a place learning memory system

Authors: *A. ALMEY, K. BERTRAM, E. CANNELL, W. G. BRAKE;
Concordia Univ., Montreal, QC, Canada

Abstract: There are at least two memory systems that rats use to learn spatial tasks such as a maze: a place memory system, where spatial cues are used to arrive at the desired location, and a response memory system, where the same physical response (i.e. turning left) is used to arrive at the desired location. Estrogens affect which of these memory systems is employed, with higher levels of estrogens biasing rats towards use of a place memory system. The hippocampus and dorsal striatum have been implicated in these memory systems but it is still not fully established where in the brain estrogen acts to bias memory system use. There is evidence that the prefrontal cortex plays an important role in solving maze tasks and the development of habitual behaviour. Therefore, this study examined whether increasing estradiol (E2) in the medial prefrontal cortex (mPFC) biases rats towards use of a place memory system. For this experiment 29 female rats were ovariectomized and implanted with bilateral cannulae; 14 had cannulae implanted in the mPFC and 15 had cannulae implanted in a control brain region, the anterior cingulate cortex (AC). The rats were trained, via 10 daily trials, to go to the same arm of a t-maze to receive a food reward. Once a rat had learned to go to the correct arm they were tested to determine what memory system they were using to navigate the maze by rotating the maze 180 degrees for testing. For the test, if the rat arrived at the arm containing the food reward this indicated that the

a place memory system was used, but if the rat arrived at the arm without the food reward this indicated a response memory system was used. Following the first test all rats were retrained, and retested following a microinfusion of either E2 or cyclodextrin, whichever they had not received prior to the first test. The results show that following an infusion of E2 to the mPFC 85% of the rats used a place memory system to navigate the maze. In contrast, 28% of rats that received cyclodextrin in the mPFC, and less than 20% of rats that received either E2 or cyclodextrin in the AC, used a place memory system to navigate the maze. This demonstrates that increasing levels of E2 in the mPFC biases female rats towards use of a place learning memory system.

Disclosures: A. Almey: None. **K. Bertram:** None. **W.G. Brake:** None. **E. Cannell:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.24/JJJ67

Topic: F.02. Animal Cognition and Behavior

Support: CNRS

Title: Role of Dopamine in observational learning in rats

Authors: *M. A. MAHMOUD[1], A. GHESTEM[1], P. CARLIER[1], M. MOFTAH[2], D. BOUSSAOUD[1];
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Abstract: Learning new sensorimotor skills relies heavily on practice, also termed trial-and-error learning (TE). In social species, TE learning may gain in speed if the learner had a chance to observe a conspecific perform the task to be learned. At the behavioral level, this gain from observation has been well demonstrated in various species, including rodents and human and non human primates. By contrast, the neural mechanisms of observational learning (LeO) remain unknown. Recent brain imaging studies in humans (Monfardini et al. unpulished) suggest that LeO and TE activate overlapping neuronal networks, including frontal cortex and basal ganglia. In monkeys, neuronal recordings have shown that prefrontal cortex neurons process feedback signals in both conditions (Isbaine et al., 2012). As dopamine plays a key role during TE learning (Puig & Miller, 2012), we hypothsized that it may also be involved in LeO. To test this hypothesis, we blocked dopamine D1 receptors (D1R) in prefrontal cortex of rats, and measured their gain from observation as compared to controls. The testing apparatus is composed of two

compartments separated by a partition, which allows visual/tactile communication between animals. One compartment (actor) contained a vertical lever which can be pushed in two directions (right or left), and a food well located at one of the corners. The correct lever push triggers the delivery of food pellets in the food well (reward), synchronized with a low frequency tone, whereas the incorrect push triggered a high frequency tone, and no reward. A group of rats were first exposed to an expert rat pushing the lever in the correct direction before they were placed in the actor compartment. In another group, rats learned through TE, and the results show that LeO rats learn much faster compared to TE rats, confirming the idea that rats can learn by observation. We then injected 2 key areas, orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) with a D1R antagonist (SCH 23390) in naïve rats before each session. Their learning rate was compared to rats injected with saline. Preliminary data show that blockade of D1R in OFC and ACC impairs severely observational learning. They suggest that dopamine plays a key role in OFC and ACC neural processes involved in observational learning.
Puig MV and Miller EK. Neuron 74, 874-886, 2012.
Isbaine F et al. Abstracts of the Society for Neuroscience. New Orleans, LA, 2012.

Disclosures: M.A. Mahmoud: None. **A. Ghestem:** None. **P. Carlier:** None. **M. Moftah:** None. **D. Boussaoud:** None.

Poster

287. **"Cognition, Learning, and Memory: Neuropharmacology"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 287.25/JJJ68

Topic: F.02. Animal Cognition and Behavior

Support: Max Planck Society support for JS

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Title: Prevention of chronic traumatic encephalopathy (CTE) by the glutamate receptor antagonist NitroMemantine in a mouse model of mild TBI

Authors: *J. SPIESS[1,2], C. WEISS[3], P. H. RIGBY[4], E. MASLIAH[5], A. M. WYRWICZ[6], J. F. DISTERHOFT[3], S. A. LIPTON[1];
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Neurosciences, UCSD, San Diego, CA; [6]NorthShore Univ. Hlth. System, Ctr. for Basic MR Research, Evanston, Il, Univ. of Chicago, Chicago, IL

Abstract: We reported earlier that we induce mild Traumatic Brain Injury (TBI) by blasting anesthetized male 8 week old C57BL/6N mice (Harlan) with a Helium pressure wave delivered from a 6 foot-long and 2 inch-wide shock tube to the back of the mouse head. Other parts of the body are protected by a firm ballistic Nylon coat. The pressure wave resembles a characteristic free field blast wave with an almost instantaneous rise to an overpressure (16 psi) lasting 1.4 msec. The blasted mice are subjected to auditory trace or delay fear conditioning as models for declarative and non-declarative memory, respectively. After a 24 hour delay, the blasted mice show increased freezing during re-exposure to the original and to a novel context which we ascribe to effects associated with Post-Traumatic Stress Disorder (PTSD). We also find decreased freezing to the auditory cue in a novel context. Behavioral changes are significantly correlated with fractional anisotropy values determined with *ex vivo* Diffusion Tensor Imaging (DTI) of the corpus callosum.

This mouse model of mild TBI has now been further analyzed for its relationship to CTE, a pathological condition that has been observed in Iraqi and Afghanistan war veterans suffering from PTSD. It has been speculated that CTE is involved in the recently increased suicide rate among veterans of the Iraqi and Afghanistan wars as well as among NFL football players. CTE is immunohistologically identified by typical cortical foci of hyperphosphorylated tau protein (phospho-tau), a CTE biomarker.

We report here that we found increased phospho-tau staining with the mouse monoclonal antibody PHF1 (Peter Davies) in slices of frontal cortex and the dentate gyrus of the hippocampus from mice that were blasted at 16 psi. The phospho-tau staining was significantly reduced when NitroMemantine, an uncompetitive dual-site NMDA-type glutamate receptor antagonist, developed in our laboratory (by SAL), was administered i.p. 30 minutes before the blast at a dose of 28 mg/kg.

We conclude on the basis of these preliminary data that NitroMemantine may qualify as a drug to ameliorate CTE and possibly mild TBI.

Disclosures: J. Spiess: None. **C. Weiss:** None. **P.H. Rigby:** None. **E. Masliah:** None. **A.M. Wyrwicz:** None. **J.F. Disterhoft:** None. **S.A. Lipton:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.01/JJJ69

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG030646

Title: Effects of nicotine exposure termination and subsequent nicotine administration on responding for delayed rewards

Authors: *K. YONEZAKI[1], J. R. FADEL[2], J. A. BURK[3];
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Abstract: Changes in delay discounting performance are known to be associated with subsequent drug use. However, the effects of abstinence and re-exposure to nicotine on sensitivity to reward contingencies are not well-characterized. In the present experiment, rats were trained in a delay discounting task and then exposed to nicotine (0.4 mg/kg, twice per day) or saline for four days. All rats then received nicotine (0.1 mg/kg) on Days 5, 8 and 12 and saline on all intervening days prior to task performance. Nicotine exposure elevated omission rates during the initial administration period. There were no differences on Day 5 between nicotine- and saline-pre-exposed rats following 0.1 mg/kg nicotine or upon termination of nicotine exposure (Days 6 & 7). Following 0.1 mg/kg nicotine on Day 8, nicotine pre-exposed rats were more likely to enter the delayed, larger reward compared with the immediately accessible smaller reward. Following saline exposure on Days 9-11, nicotine pre-exposed animals were less likely to enter the delayed, larger reward and omitted more trials than saline pre-exposed rats. Following 0.1 mg/kg nicotine on Day 12, the group differences in choice preference and omissions were no longer present. Water consumption from the home cage measured after completion of delay discounting testing was not affected by prior repeated nicotine exposure. Termination of nicotine exposure and subsequent nicotine re-exposure may decrease sensitivity to reward contingencies which could increase risk of future drug use.

Disclosures: K. Yonezaki: None. **J.R. Fadel:** None. **J.A. Burk:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.02/JJJ70

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG030646

Title: Loss of medial prefrontal cortical cholinergic projections increases preference for an immediately available reward in a delay discounting task

Authors: *J. A. BURK[1], K. YONEZAKI[2];
[1]Psychology, Col. of William & Mary, Williamsburg, VA; [2]Hokkaido Univ., Yokohama, Japan

Abstract: Attentional control is thought to regulate numerous processes, including impulsive choices. The cholinergic projections to the medial prefrontal cortex are thought to be part of a distributed neural circuit that maintains attentional control. In the present experiment, male FBNF1 hybrid rats were trained in a delay discounting task that involved a choice between a small, immediately available reward and a larger reward. The larger reward was also immediately available at the beginning of the test session and then the delay to receive this reward was progressively increased within a session (0-40 s). After reaching stable performance levels, rats then either received infusions of the cholinergic immunotoxin, 192IgG-saporin, or vehicle into the medial prefrontal cortex. After recovering from surgery, rats were tested in the same delay discounting task that had been trained prior to surgery. Relative to sham-lesioned animals, rats with a loss of the cholinergic projections to the medial prefrontal cortex exhibited an increased preference for selecting the smaller, immediate reward. Subsequent administration of nicotine (0.0, 0.1, 0.2, 0.4 mg/kg, ip) did not substantially alter the effects of the lesion on delay discounting performance. The present results suggest that medial prefrontal cortical cholinergic projections contribute to choice behavior based upon delay to reward access and reward magnitude. Moreover, these results are consistent with the idea that disruption of attentional control can increase impulsive choices.

Disclosures: J.A. Burk: None. **K. Yonezaki:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

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Program#/Poster#: 288.03/KKK1

Topic: F.02. Animal Cognition and Behavior

Support: NIH SC2MH087974

Title: Analysis of the use of positive and negative feedback after methamphetamine

Authors: *A. STOLYAROVA, D. RODRIGUEZ, A. IZQUIERDO;
California State Univ, Los Angeles, Los Angeles, CA

Abstract: Convergent evidence from rat, monkey, and human studies suggests maladaptive decision making in methamphetamine (METH) addiction. Actor-Critic architecture is currently a widely accepted model of striatal function, in which ventral striatum is assigned the role of critic, which stores state-dependent values and signals reward prediction error (δ), and dorsal striatum represents an actor, which assigns state-action-dependent preferences (Sutton & Barto, 1998; Takahashi et al. 2008). Reversal learning is a behavioral task measuring flexibility of response to changes in reward contingency and is well-suited to examine animals' ability to integrate positive and negative outcomes into decision making within the framework of the actor-critic model. In the present experiment we compared the long-term effects of METH and saline (SAL) on pairwise visual discrimination reversal learning. Specifically, we analyzed how animals use positive and negative trial-by-trial feedback (Izquierdo et al. 2013), domains not previously explored in rat drug studies. No significant treatment group differences were observed in overall trial-by-trial responses to positive or negative feedback in reversal learning. However, based on previous suggestions that task demand and interference strength change across stages of reversal learning, we performed analyses of 3 distinct stages: zero to 50% correct (Stage 1), at 50% (Stage 2) and 50% to criterion (Stage 3). Trial-by-trial analysis showed that METH-treated animals benefited more from correctly-performed (C) trials Δ(C+1)/C total during Stage 1 and required more experience switching from incorrectly-performed (E) to C trials Δp(E+1)/ΔpC to update behavioral responses compared to SAL. Differences in learning from C feedback persisted into Stage 2. SAL-treated animals' progression to Stage 3 was accompanied by a reduction in the proportion of C trials resulting from negative feedback Δ(E+1)/C total, whereas METH-treated animals failed to demonstrate the same pattern. Prolonged escalating METH exposure, not binge exposure, yielded the most aberrant responses. Binge-treated rats, however, showed less reduction in C trials followed by E trials Δ(C+E)/C total. No differences were observed in response to either C or E feedback across groups at Stage 3. Overall, results indicate enhanced learning from positive feedback and increased interference in METH treated rats. This suggests both increased positive δ and decreased rate of state value update in the Actor-Critic after METH. Additional experiments are aimed at uncovering the neural correlates of these maladaptive learning patterns.

Disclosures: A. Stolyarova: None. **D. Rodriguez:** None. **A. Izquierdo:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

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Program#/Poster#: 288.04/KKK2

Topic: F.02. Animal Cognition and Behavior

Support: NIH SC2MH087974

Title: Discrimination reversal learning and self-administration in adulthood following adolescent methamphetamine exposure

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Abstract: Adolescence is a period of profound neurobiological change. As the frontal cortex continues to develop through adolescence into adulthood, the ongoing refinement of frontocortical processes (i.e., cognitive control) precipitates heightened impulsivity and poor decision-making. Poor cognitive control in adolescence may contribute to an accelerated progression from recreational use to compulsive substance use in adulthood. Previous research found increased vulnerability for learning and memory impairments when rats were exposed to methamphetamine (METH) in late adolescence, specifically Post Natal Days (PND) 41-50 (Vorhees et al., 2005) and PND 50-51 (White et al., 2009). The purpose of the present study was to investigate the long-term effects of escalating, adolescent METH exposure on cognitive flexibility as measured by visual discrimination and reversal learning in adulthood (Experiment 1) and the likelihood of later self-administration of the drug (Experiment 2), also in adulthood. In Experiment 1, male Long-Evans rats were treated with either saline (n=8 SAL) or escalating doses of 0.3 mg/kg to 3.0 mg/kg METH during PND 41-50 (n=10 METH). Following treatment, rats were trained to nosepoke stimuli associated with reward (S+) and withhold response to stimuli associated with no reward (S-) on a touchscreen, i.e. visual discrimination learning. Upon reaching criterion, reward contingencies were reversed, i.e. reversal learning. Results showed there was a significant effect of METH treatment on both visual discrimination and reversal learning, with most pronounced attenuations in the reversal learning phase. Impairments in this phase are consistent with previous research in the adult, however, discrimination learning impairments have not been previously reported. In Experiment 2, rats exposed in adolescence to either METH or SAL underwent voluntary self-administration of low (10 mg/l) and high dose (20 mg/l) METH or quinine in a two-bottle choice design: a bottle of pure water vs. a bottle mixed with METH or quinine. We found that rats pre-exposed to METH during adolescence self-administered significantly more drug in adulthood. Additionally, results showed there was a significant positive correlation between sessions to criterion on discrimination learning and consumption of the low METH dose. Taken together, these findings show that even modest exposure to METH during late adolescence may induce long-term general learning impairments and an increased likelihood to self-administer METH in adulthood. Ongoing experiments are aimed at uncovering the neural correlates of these behaviors.

Disclosures: T. Ye: None. **H. Pozos:** None. **T.J. Phillips:** None. **A. Izquierdo:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.05/KKK3

Topic: F.02. Animal Cognition and Behavior

Support: The Wellcome Trust (Grant 082535/Z/07/Z)

The intramural research program of the National Institute of Mental Health

Title: Effects of a D2R antagonist on changes in cross-frequency power coupling related to the trade-off between attention demanding and automatic processes in frontal-striatal circuits

Authors: *E. LEE[1], M. SEO[1,2], O. DAL MONTE[1], B. B. AVERBECK[1];
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Abstract: In daily life, we often shift between automatic and attention demanding processes without effort. Emerging research suggests that frontal-striatal circuits are involved in the trade-off between attention demanding and automatic processes. However, the role of dopamine in frontal-striatal circuits in this trade-off has not been examined directly. To investigate this, we trained monkeys on an oculomotor sequential decision making task with random and fixed conditions. In the random condition (attention demanding) the correct spatial sequence of eye movements varied from trial to trial and the animal had to carry out a difficult perceptual decision to determine where to saccade. In the fixed condition (automatic), the sequence was fixed for blocks of eight correct trials. Thus once the animal learned the sequence it could automatically execute it from memory. While the monkeys performed the task, we injected locally a dopamine D2R antagonist (Eticlopride, 1.2ug/1ul) or saline into the dStr (2ul/site at 3nl/sec, 8 ul/side) bilaterally. Moreover, we recorded local field potentials from lateral prefrontal cortex (lPFC) and dorsal striatum (dStr) simultaneously with the injections.
We examined changes in cross-frequency power coupling between pre- and post-drug (saline) injections within each session. DStr-dStr cross frequency power coupling showed significant changes in the 8-30 hz (alpha and beta) range during the task in the fixed condition in drug sessions. LPFC-dStr power coupling showed significant changes in the 13-30hz (beta) range during the stimulus presentation period and the 8-12hz (alpha) range during the movement period in the fixed condition in the drug sessions. In saline sessions, no changes were found between the pre and post injection periods. Overall, our results suggest that the indirect pathway through the basal ganglia, which contains primarily D2Rs, modulates the transition between attention demanding and automatic processes. Furthermore, the effects of antagonist injections on the interaction within the dStr and between dLPFC-dStr in the 8-30 hz (alpha and beta) range

suggests an important role for dopamine in the trade-off between attention demanding and automatic processes.

Disclosures: E. Lee: None. **M. Seo:** None. **O. Dal Monte:** None. **B.B. Averbeck:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.06/KKK4

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01AA022249

Title: Increased impulsive action after a history of chronic alcohol intake and abstinence

Authors: *C. IRIMIA, I. Y. POLIS, L. H. PARSONS;
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Abstract: Impaired cognitive processing is a hallmark of addiction. In particular, deficits in inhibitory control (impulsive action) can propel continued drug use despite adverse consequences. Clinical studies have provided substantial evidence that detoxified alcoholics exhibit poor inhibitory control in the Continuous Performance Task (CPT) and related tests of motor impulsivity. Premorbid impulse control disorders also contribute to the initiation of problem drinking, for example adolescents that score higher on the Baratt Impulsivity Scale are also more likely to use alcohol and other drugs and to start drinking at an earlier age. A persistent question in alcohol research regards the relative influence of pre- existing cognitive disruptions that confer susceptibility to problem alcohol use versus the induction of cognitive impairment related to cortical damage induced by repeated cycles of intoxication and withdrawal. In this regard animal models can provide important insight into the etiology of alcohol-induced cognitive impairment and can provide a platform for mechanistic studies and rapid pharmacotherapy screening. Using behavioral paradigms analogous to clinically employed tasks we have gathered evidence of significant increases in impulsive action that emerge during protracted withdrawal in alcohol-dependent rats. In the 5 Choice Serial Reaction Time Task (5CSRTT) we observe an emergence of significant impulsive action behavior over the course of 34d of abstinence. This form of impulsivity is ameliorated following a return to alcohol consumption, but re-emerges during subsequent periods of abstinence. Using a novel animal model of CPT we find that alcohol-dependent rats also exhibit an inability to suppress prepotent responses (e.g. high levels of false alarms) during early stages of withdrawal along with excessive premature responding under task conditions with enhanced cognitive load, and these

disruptions persist during at least 7 weeks of abstinence. These forms of impulsive behavior are reliant on proper function of the medial prefrontal cortex, and as such our findings point to dependence-induced impairment of frontal cortical function. Collectively, these findings demonstrate the emergence of increased impulsive action in alcohol-dependent rats during protracted withdrawal and this may provide a viable animal model for characterizing the neurobiological substrates underlying dependence-related impulsivity.

Disclosures: C. Irimia: None. **L.H. Parsons:** None. **I.Y. Polis:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.07/KKK5

Topic: F.02. Animal Cognition and Behavior

Support: NIH RO1 DA 027222

Title: Adolescent behavior and nac neuronal recording following methylphenidate (0.6, 2.5, 5.0, 10.0 mg/kg i.p.)

Authors: A. FROLOV[1], *C. REYES-VAZQUEZ[2], N. DAFNY[1];
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Abstract: Methylphenidate (MPD) has become one of the most commonly prescribed drugs for the treatment of ADHD. The broadening of its use both as a standard treatment and, more controversially, as a method of cognitive enhancement and recreation has led to a need to evaluate its effects more closely, especially when used chronically in adolescents. Studies have demonstrated that after chronic administration of the same dose of MPD, some animals will exhibit behavior indicative of tolerance to the drug, while others will exhibit behavior indicative of sensitization. Furthermore, the nucleus accumbens (NAc) has been shown to play a key role in the brain's reward response to MPD, and thus is the target of this study. The present study postulates that the same dose of MPD will elicit in some animals behavioral sensitization and in others tolerance, and that NAc neuronal activity from animals expressing behavioral tolerance will be significantly different from NAc neuronal activity recorded from animals expressing behavioral sensitization. To test this hypothesis, behavioral and neuronal activity was recorded from the NAc of freely behaving adolescent rats before and after acute and chronic administration of 4 different MPD doses. Five groups of rats were used: all received saline injections, followed by either saline or 0.6, 2.5, 5.0, or 10.0 mg/kg MPD i.p. on experimental day

1 (ED1). On ED2 to ED6, the control group received saline, while the others received the same dose of MPD as they did on ED1. On ED7 to ED9, the rats received no injections, representing a washout period. Finally, on ED10, neuronal activity was recorded after saline or MPD rechallenge in the same manner as on ED1. Statistical analysis of the data reveals that at all doses of MPD (but not in saline controls), some animals demonstrate behavioral tolerance and others behavioral sensitization, and that neuronal firing rates in animals exhibiting sensitization show a significantly different response to MPD from neuronal firing rates in animals exhibiting behavioral tolerance. This indicates that when studying the effects of chronic MPD on adolescents, it is critical to simultaneously record both neuronal activity and behavioral data, and to evaluate all data based on the animals' behavioral response to chronic MPD exposure.

Disclosures: A. Frolov: None. **C. Reyes-Vazquez:** None. **N. Dafny:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.08/KKK6

Topic: F.02. Animal Cognition and Behavior

Support: CNPq

Title: Etanol,exposure in binge episodes during adolescence change the bdnf levels, attention and aggressive behavior in adults wistar rats

Authors: *R. M. DE ALMEIDA[1], L. BIZARRO[1], L. SCHEIDT[1], A. PIRES[1], J. PASSOS[1], L. STERTZ[2], G. FRIES[2], F. KAPCZINSKI[2], K. MICZEK[3];
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Abstract: Animal models provide a useful alternative for studying the mechanisms of dependence and cognitive-behavioral deficits caused by repeated by alcohol intake during adolescence. Morphological and molecular changes in the limbic system such as hippocampus have been documented after many types of stress including alcohol administration. We investigated the effects of ethanol exposure in adolescent rats during adulthood and assesss the attention, impulsivity, aggression, anxiety-like behaviors and the check the levels of inflammatory markers. Three groups of male Wistar rats (mean weight 81.4 g, N=120 were housed in groups of four until post-natal day 60 (P60). From days P30-P46 rats received one of three by ">treatments: 3 g/kg of ethanol (15% v/v, PO, n = 48), 1.5 g / kg of ethanol (12.5% v/v, PO, n = 36), or water (n = 36) every 48 hours. Groups of 40 animals were studied for aggression

and anxiety-like behaviors; attention and impulsivity. And also some animals were assessed to investigated the levels of BDNF on the pre-frontal cortex and hippocampus. After P60, attentional
performance was assessed in the 5-choice serial reaction time task (5CSRTT) and impulsivity was assessed on an operant delay discounting task. Results: In the 5-CSRTT, animals achieved stable base line responding in 89 sessions, with no differences among treatment groups. In test sessions, when the ITI was 15s the group that received 3 g/kg showed lower accuracy (72.5%) than the others (81.9% accuracy in the 1.5 g/kg group and 83.4% in the water group). The latency of correct
responses was longer in 1.5 g/kg group than the latency of 3 g/kg group ($p < 0.05$). There was no group effect or group x session type interaction in the other measures. With respect to delay discounting, the animals treated with alcohol (1.5 g/kg and 3.0 g/kg) did not show any difference when compared to control group. Animals treated with the lower concentration of alcohol were more aggressive (i.e. showed shorter attack latency to attack the intruder) when compared to the control group and had no effects on anxiety-like behaviors on the EPM as compared to control group. The animals wich receive a low concentartion of alcohol had low levels of BDNF in the hippocampus but not prefrontal cortex when compared to the control group.Binge-like, intermittent exposure to alcohol during adolescence led to impaired performance on an attention task in adulthood and may have compromised the readiness to respond. Adult animals were more promp to attack the intruder, showing more aggressive behaviors.
These behavioral aberrations were accompained by decreased level of BDNF in the hippocampus.

Disclosures: R.M. De Almeida: None. **L. Bizarro:** None. **L. Scheidt:** None. **A. Pires:** None. **J. Passos:** None. **L. Stertz:** None. **G. Fries:** None. **F. Kapczinski:** None. **K. Miczek:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.09/KKK7

Topic: F.02. Animal Cognition and Behavior

Support: NIDA Grant R01DA027688

Title: The contribution of nicotinic acetylcholine receptors to learned inhibition

Authors: R. B. PUTNEY, *D. J. BUCCI;
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Abstract: Learning to withhold a behavioral response when signaled by a cue in the environment (i.e., a 'stop signal') is an essential aspect of cognitive control and inhibition. Compared to adults, adolescent humans and laboratory animals often exhibit difficulty in using stop signals to guide behavior and successfully withhold a response and these deficits are exacerbated in ADHD and schizophrenia. Adolescents are also particularly vulnerable to taking up cigarette smoking, and nicotine use in persons with ADHD or schizophrenia is disproportionately high compared to the general population. Recent studies have shown that nicotine administration in juveniles and young adults with ADHD normalizes deficits in behavioral inhibition as measured in the Stop Signal Reaction Time Task. We have previously modeled this finding in rats by showing that treatment with nicotine enhances negative occasion setting, a form of learned inhibition. In this procedure, a tone is presented and followed immediately by food reward on a subset of trials. On other trials, a light precedes the tone and no food is delivered. Normal adult rats learn to discriminate between these two trial types after ~7 training sessions, indicated by approaching the food cup in anticipation of receiving a reward when the tone is presented by itself, but inhibiting that response when the light precedes the tone. Nicotine (0.35mg/kg free base equivalent) reduces the number of sessions needed to learn the discrimination and also enhances the magnitude of discrimination between trial types specifically by reducing responding during the non-reinforced trials. Here we tested additional doses of nicotine to derive a dose response curve and also tested the effects of mecamylamine (nicotinic acetylcholine receptor antagonist) to determine whether cholinergic activity is necessary for negative occasion setting. Adult male Long-Evans rats were maintained at 85% free feeding body weight and treated with either mecamylamine (0.125-2.0 mg/kg) or nicotine (0.2-0.5 mg/kg, free base equivalent) 20 minutes before each daily training session. Compared to vehicle-treated control rats, those treated with mecamylamine needed more sessions to learn the discrimination and also did not exhibit as robust discrimination between the trail types. Rats treated with nicotine learned the task in fewer sessions in a dose-dependent fashion. Together with the finding that rats with lesions of the prefrontal cortex are impaired in negative occasion setting, these data indicate that acetylcholine, perhaps acting through cortical nicotinic receptors, may be necessary for learning to inhibit behavioral responses.

Disclosures: R.B. Putney: None. **D.J. Bucci:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

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Topic: F.02. Animal Cognition and Behavior

Support: NIH MH093354-01A1

Alz Assoc NIRG-11-203107

Ellison/AFAR Postdoctoral Fellows in Aging Research Program

Title: Nicotinic α4β2 receptor stimulation strengthens working memory-related neuronal activity in prefrontal cortex

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Abstract: Acetylcholine acts through a variety of nicotinic and muscarinic receptors to modulate wakefulness, and is essential for the working memory (WM) functions of the primate dorsolateral prefrontal cortex (dlPFC). Our previous study showed that nicotinic α7 acetylcholine receptors (α7-nAChRs) are localized in dlPFC synapses on spines, and are essential for the persistent neuronal firing of dlPFC circuits, permitting the NMDAR actions needed for communication between dlPFC neurons. Nicotinic α4β2 receptors are another important nicotinic receptor subtype in PFC. There is behavioral evidence that α4β2 stimulation can improve the working memory and attention functions of the PFC in rodents, monkeys and humans. The current study examined the effects of α4β2 receptor stimulation on the persistent neuronal firing of neurons in the dorsolateral PFC in monkeys performing a spatial working memory task. α4β2 receptor agonists were applied directly onto the neurons using iontophoresis. Preliminary data show that iontophoresis of the α4β2 receptor agonist, ABT-418, produces a dose-related enhancement of delay-related persistent activity and improvement in spatial tuning. These results indicate that an α4β2 receptor agonist, similar to an α7 receptor agonist, can enhance the memory-related firing of the dlPFC, encouraging the development of α4β2 receptor agonists as potential treatments for cognitive disorders.

Disclosures: Y. Yang: None. **M. Wang:** None. **L.E. Jin:** None. **A.F.T. Arnsten:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust Programme Grant (089589/z/09/z)

Joint MRC and Wellcome Trust BCNI Centre Grant G1000183

Title: Investigating the role of striatal dopamine signalling during performance of a visual discrimination reversal task

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[1]Dept. of Psychology, [2]Behavioural and Clin. Neurosci. Inst., [3]Dept. of Physiology, Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Behavioural flexibility is impaired in numerous neuropsychiatric conditions, including Obsessive Compulsive Disorder (OCD; Dalley et al., Neuron, 2011). Reversal learning is a test of behavioural flexibility in which the stimulus-outcome contingencies switch, such that subjects must inhibit responding to previously rewarded stimuli and direct actions toward stimuli with no immediate history of reward. Previous studies in marmoset monkeys have demonstrated an important role for orbitofrontal cortex (OFC; Dias et al., J Neurosci, 1997) and medial striatum (Clarke et al., J Neurosci, 2008) in reversal performance. Notably, these regions have also been shown to be hypoactive during reversal learning in OCD patients (Remijnse et al., Arch Gen Psychiatry, 2006). Dopamine (DA) is a prominent neuromodulator in the striatum and is thought to be involved in facilitating learning about new or changing contingencies between behavioural events and outcomes (Schultz & Dickinson, Ann Rev Neurosci, 2000). Indeed, depletion of DA in marmoset caudate has been shown to produce deficits when stimulus-outcome contingencies are reversed (Clarke et al., J Neurosci, 2011). Recent studies have revealed a specific role for DA D2 receptors (D2Rs) in these effects. Relatively lower levels of D2Rs in the caudate have been linked to reversal deficits in human subjects (Jocham et al., J Neurosci, 2009) and vervet monkeys (Groman et al., J Neurosci, 2011) and are evident in OCD patients (Denys et al., Biol Psychiatry, 2009). To further examine the role of striatal D2Rs in flexible behaviour, we trained marmosets in a serial reversal task in which stimulus-outcome contingencies were switched within-session. Cannulae were implanted bilaterally in ventromedial caudate, which receives dense projections from OFC (Roberts et al., J Comp Neurol, 2007). Reversible inactivation of this region by co-infusion of the GABA-A agonist muscimol (2.2 nmol / side) and the GABA-B agonist baclofen (2.2 nmol / side) disrupted reversal learning in two animals, measured as an increase in trials to criterion greater than the 95% confidence interval of baseline responding. Local infusions of quinpirole (0.3-10.0 µg / side), a D2R agonist, improved reversal at moderate doses and impaired reversal at high doses. These manipulations did not affect retention. Saline infusions had no effect. These data confirm that the ventromedial caudate is required for optimal reversal performance in marmosets. The data also indicate that there is an inverted U-shaped

relationship between D2R stimulation and performance, which will be discussed in terms of pre- vs. post-synaptic D2R function (Cools et al., J Neurosci, 2009).

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Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

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Topic: F.02. Animal Cognition and Behavior

Support: DA026472

AA0188330

VA Merit Award

Title: Regional differences in amphetamine-induced dopamine release in non-human primates

Authors: *H. P. JEDEMA[1], R. NARENDRAN[2], C. W. BRADBERRY[3,4];
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Abstract: Amphetamine (AMPH) is frequently used to increase catecholamine levels in attention disorders and PET imaging studies. Although the effect of AMPH varies across regions in rodents, data on regional differences in the primate are very limited, despite their frequent use to characterize PET ligands, including novel ligands whose displacement can be measured in the cortex (Narendran et al., 2013). The present study examined the impact of AMPH on the extracellular levels of dopamine (DA) in the prefrontal cortex and the caudate of non-human primates (n=5).
Average baseline levels in the anterior cingulate cortex were 0.31±0.03 nM (n=31 observations). Upon AMPH injection (0.15, 0.3, 0.5, or 1.0 mg/kg) cortical DA levels increased to 593±36, 965±130, 1385±213, or 2067±393 % of baseline levels, respectively. Peak DA levels in the cortex varied linearly with dose and were achieved on average 53±4 min after AMPH. Average

baseline DA levels in the caudate were 12.9±2.9 nM (n=12 observations). AMPH injection (0.3, 0.5, or 1.0 mg/kg) increased DA levels in the caudate to a greater extent, 1834±282, 2670±809, or 5807±235 % of baseline values, respectively. Peak DA levels in the caudate were achieved significantly faster, on average 32±3 min after AMPH. DA levels in the caudate appeared to decline in a biphasic manner, with an initial phase of rapid decline followed by a phase of slower decline similar to the steady decline observed in the cortex. Extrapolation of the data using first order kinetics over the final 70 min of the experiment (50-110min post AMPH) yielded a half-life of approximately 2.5±0.5 and 1.6±0.5 hrs in the cortex and caudate, respectively. These regional differences in amplitude and temporal profile of AMPH-induced DA levels can likely be attributed to differences in the regulation of DA uptake and biosynthesis. Furthermore, these regional differences in DA response may provide insight into the therapeutic use of stimulants in attention disorders and may have implications for the interpretation of PET and pharmacological MRI studies.

Disclosures: H.P. Jedema: None. **R. Narendran:** None. **C.W. Bradberry:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

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Topic: F.02. Animal Cognition and Behavior

Support: NSF-1121147

Klarman Family Foundation

Title: Neural control of feeding: Optogenetic and pharmacological studies on the medial frontal cortex and ventral striatum

Authors: *M. A. PARENT[1], M. LAUBACH[2];
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Abstract: Previous work from our lab demonstrated a role of the medial frontal cortex (MFC) in control of feeding (Parent et al., SFN 2012, 604.03). Rats were trained to perform a consummatory incentive contrast task and received alternating 30 seconds access to high value (20% sucrose) or low value (4% sucrose) solutions. Animals learned to inhibit their drinking during low value epochs and augment their drinking during high value epochs to maximize reward. We originally hypothesized that inactivation of MFC would disrupt the ability to inhibit

consumption of low-value solutions. However, using muscimol-based inactivations, we found that MFC was instead crucial for the maintenance of bouts of licking and did not lead to overconsumption of the low-value solution. Here, we report further studies on this issue using targeted pharmacological and optogenetic inhibition of MFC. We found that intake is differentially influenced by inactivation across the rostral-caudal axis of MFC, with consumption of high value sucrose affected to a greater degree with inactivation of the rostral part of MFC. Previous recordings of unit activity and local field potentials in MFC show that spike activity is modulated, and field potentials show phase locking, around bouts of feeding (Horst and Laubach, 2013). Classic studies on cortical oscillations have implicated the cholinergic system in the control of cortical rhythms. Therefore, we examined how cholinergic neuromodution of MFC influences intake behavior, especially the microstructure of licking. Infusions of the muscarinic antagonist, scopolamine, into MFC dramatically decreased consumption of the high value option, paralleling the effects of MFC inactivation. Oppositely, XE991, an M-current blocker whose underlying channel is controlled by muscarinic receptors, increased the consumption of high value solution. Due to the heavy efferent projections of MFC to ventral striatum (VS), and in line with previously published work (Baldo et al., 2005), we explored the role of VS in consummatory contrast and found that inhibition of VS increased consumption in the contrast task. This suggests that interactions between MFC and VS have opposing roles in the control of feeding. We propose that cholinergic-sensitive oscillatory neuronal activity in the MFC is crucial for the maintenance of goal-directed action sequences (e.g. measured by the duration of bouts of feeding) and that inhibitory control over intake is based subcortically, in the ventral striatum.

Disclosures: M.A. Parent: None. **M. Laubach:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.14/KKK12

Topic: F.02. Animal Cognition and Behavior

Support: FAPESP Grant 2010/11588-0

Title: Concomitant apomorphine and cholecystokinin supress apomorphine tolerance induction

Authors: *C. A. TIEPPO[1], H. R. SILVA[2], C. LOPES[3];
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[3]Physiological Sci. Dept., Santa Casa of Sao Paulo Med. Sci. School, Sao Paulo - Brazil, Sao Paulo, Brazil

Abstract: Objective: Chronic apomorphine (APO) treatment, a non-specific dopaminergic agonist, induces a classical behavioral tolerance to APO. Cholecystokinin (CCK) treatment modifies behavioral responses related to dopaminergic stimulation. Our previous results showed that CCK was able to decrease APO-induced stereotyped behavior scores in rats. Our goal was to test if CCK associated with APO chronic treatment can modulate stereotyped behavior responses to APO.
Methods: Eighty young male Wistar rats with approximately 280 g were divided in eight groups and submitted to a seven-days APO treatment (n=40; 0.6 mg/kg; s.c., once a day) or saline (n=40). Fifteen min before APO, animals received CCK4 (12 nmol/kg; i.p.), CCK8S (12 nmol/kg; i.p.) or saline (SAL). On the 8th day, all animals received APO (0.6 mg/kg; s.c.) and the stereotypy was immediately evaluated by Setler's score scale (10 s were observed in every 10 min, during 60 min) as follows: 0 - asleep or still; 1 - active; 2 - predominantly active but with bursts of stereotyped sniffing and rearing; 3 - constant stereotyped activity, but with locomotor activity still present; 4 - constant stereotyped activity maintained at one location; 5 - constant stereotyped activity with bursts of licking and/or gnawing and biting; 6 - continual licking of cage grids. Setler scores were analyzed by Mann Whitney U test. Animal Experimentation Ethics Committee protocol number: CEUA 011/09.
Results: The seven-days APO treatment induced dopaminergic tolerance, as rats treated with SAL and APO displayed less intense stereotyped behavior expression (SAL-APO: 16 {14;17}) when compared to SAL-SAL group (21 {19;25}), $p<0.0001$ on the 8th day of treatment. CCK-4 and CCK-8 treatments suppressed APO-induced dopaminergic tolerance, as animals treated with CCK8S-APO and CCK4-APO showed stereotyped behaviors higher than with the treatment with APO alone (CCK4-APO: 20 {19;22} and CCK8S-APO: 20 {19;24} vs SAL-APO 16 {14;17}) $p<0.006$ and compatible with those who received only SAL.
Conclusions: The APO treatment protocol used was able to induce APO tolerance. The long-term treatment with the CCK agonists prevents expression of tolerance, revealing a potential role of the CCK system in the genesis of this phenomenon. In previous results, we showed a remarkable APO tolerance after chronic cocaine treatment. It reveals the importance of a broader understanding of CCK modulation of the dopaminergic plasticity for enhance our understanding of drug addiction mechanisms.

Disclosures: C.A. Tieppo: None. **H.R. Silva:** None. **C. Lopes:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.15/KKK13

Topic: F.02. Animal Cognition and Behavior

Support: NIDA Grant DA11064

Title: 5-HT2AR antagonism and 5-HT2CR stimulation attenuates hyperlocomotion produced by intra-striatal cocaine infusions

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Abstract: We have previously shown that 5-HT_{2A} receptor (R) blockade and 5-HT_{2C}R activation interact to attenuate cocaine-induced hyperlocomotion and Fos expression in the dorsal caudate-putamen (dCPu). This study examined whether the 5-HT_{2A}R antagonist M100907 and the selective 5-HT_{2C}R agonist CP809101 attenuate hyperlocomotion produced by cocaine infusions into dCPu. We first examined the dose-dependent effects of CP809101 (0.25, 0.5, 1.0 mg/kg, SC) on spontaneous locomotion to find a subthreshold dose (i.e., the highest dose that failed to alter spontaneous locomotion). Subsequently, a dose of M100907 (0.025 mg/kg, SC) previously found to be subthreshold for altering locomotion and the subthreshold dose of CP809101 (0.25 mg/kg, SC) were used in a combined cocktail in order to examine receptor interactions locomotion produced by intra-CPu cocaine infusions (100 μg/side at a volume of 0.05 μl/side). Male rats were tested repeatedly with a minimum of 3 days between each test day under each of the following drug conditions: 1) vehicle; 2) 0.025 mg/kg M100907; 3) 0.25 mg/kg CP809101; and 4) a cocktail of the same doses of M100907 and CP809101 combined (n=15). On each test day, rats were placed into the test cage for a 1-h habituation phase. They then received the drug treatment and were returned to their home cage for 30 min. Next, rats received cocaine infusions (100 μg/side) into the dCPu and were immediately placed back into the test cage for 1 h. As expected, M100907 given alone had no effect on cocaine-induced locomotion, whereas, CP809101 given alone produced a mild attenuation. In contrast, the cocktail attenuated cocaine-induced locomotion to a greater extent than CP809101 alone (i.e., the cocktail group significantly differed from all other groups). These findings suggest that 5-HT_{2A}Rs and 5-HT_{2C}Rs do in fact interact to attenuate hyperlocomotion produced by intra-dCPu cocaine infusions. These findings provide further support for the idea that concurrent 5-HT_{2A}R antagonism and 5-HT_{2C}R agonism may offer a novel approach to treating cocaine dependence. This research was supported by National Institute on Drug Abuse (DA11064).

Disclosures: T. Der-Ghazarian: None. **L. Pockros:** None. **R. Mirando:** None. **S. Brunwasser:** None. **N. Pentkowski:** None. **J. Neisewander:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.16/KKK14

Topic: F.02. Animal Cognition and Behavior

Support: R01 MH081843-05

F31 MH098631-02

Title: Receptor and frontostriatal circuit mechanisms underlying the cognition-enhancing actions of psychostimulants

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Abstract: Psychostimulants, including Methylphenidate (MPH, Ritalin), enhance a variety of cognitive/behavioral functions dependent on the prefrontal cortex (PFC) and extended frontostriatal circuitry. These actions are seen in individuals with and without Attention Deficit Hyperactivity Disorder (ADHD) as well as normal animal subjects. Despite widespread use of these drugs, the brain regions/receptor mechanisms involved in their cognition-enhancing and therapeutic effects remain poorly understood. The current studies examined the degree to which MPH acts within distinct frontostriatal subfields to improve PFC-dependent cognition and the receptor mechanisms involved in these actions. We observed that MPH elicited an inverted-U shaped facilitation of PFC-dependent cognition when infused into the dorsomedial PFC (dorsal prelimbic and anterior cingulate; maximum improvement at 0.125 μg/500 nl), but not ventromedial PFC (infralimbic), dorsomedial striatum, or ventromedial striatum (Nucleus Accumbens). The ability of MPH to improve working memory when infused into the dorsomedial PFC was blocked with the concurrent infusion of either a NE α2 (Atipamezole; 1.25 μg/500 nl) or DA D1 (SCH23390; 0.5 μg/500nl), but not NE α1 receptor antagonist (benoxathian; 2nMol/500 nl), at doses that did not alter performance when given alone. These observations provide the first definitive evidence that psychostimulants act at NE a2 and DA D1 receptors directly within the PFC to enhance delay related cognition.
Recent evidence from our lab demonstrates that in contrast to spatial working memory tasks, performance in a sustained attention task shows a broader and right-shifted dose response curve to MPH. Furthermore, this improvement in sustained attention requires action at α1 receptors. Ongoing studies are identifying the frontostriatal circuitry and receptor mechanisms involved in attention-enhancing effects of MPH and the degree to which these mechanisms differ from the working memory-enhancing effects of this drug.

Disclosures: R.C. Spencer: None. **C.W. Berridge:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.17/KKK15

Topic: F.02. Animal Cognition and Behavior

Support: F32-DA030831

R01-MH073689

Title: Dopamine depletion in the dorsolateral striatum impairs the acquisition of an egocentric task but not spatial reversal or strategy switch

Authors: *J. J. YOUNG, K. SEIP-CAMMACK, M. L. SHAPIRO;
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Abstract: Damage to the dorsolateral striatum (DLS) in rodents and primates impairs egocentric (body-turn) and stimulus-response (S-R) learning. While dopamine is required for S-R learning, the specific mechanism supporting this type of learning remains unclear. We hypothesized that dopamine in the DLSmay help consolidate S-R reward associations and helps to transmit that information to extended memory systems (e.g., prefrontal, hippocampal) for higher-order processing. Rats receivedbilateral microinjections of6-hydroxydopamine (6-OHDA) into the DLS to selectively destroy dopaminergic (DA) input from thesubstantianigra pars compacta (SNc). Lesions eliminated approximately 50-70% of the total DA input to the DLS. After recovery from surgery, the rats were trained on an egocentric or a spatial task on a plusmaze. After reaching a criterion of six consecutive correct trials, ratswere given 24 additional trials to establish stable performance.The ratswere then trained to the same criterion on alternating spatial and egocentric tasks, which required them to switch strategies between tasks, over the subsequent 6-10 days. A separate group of rats was trained in a series of spatial reversals that did not include strategy switches.The effect of DA depletion on egocentric tasks varied with training history. Rats with DA depletion learned the egocentric taskmore slowly than intact rats only if they learned this task first. In contrast,rats with DA depletion trained first in the spatial task learned the egocentric task normally. After learning to criterion,both groupsperformed equivalently on each task. DA depletion did not impair rats' ability to switch between egocentric and spatial strategies. The transient and selective impairment in egocentric learning, together with intact strategy switching suggests that dopamine in the DLSmay contribute toinitial S-R

reward learning when extended memory systems have not already been involved in the acquisition of similar tasks.

Disclosures: J.J. Young: None. **M.L. Shapiro:** None. **K. Seip-Cammack:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R37 AG06647

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Intramural Research Program of NIA

Title: Presynaptic mitochondrial morphology in the dorsolateral prefrontal cortex correlates with working memory and is improved with estradiol treatment in rhesus monkeys

Authors: *Y. HARA[1,2], F. YUK[1,2], R. PURI[1,2], W. G. M. JANSSEN[1,2], P. R. RAPP[5], J. H. MORRISON[1,2,3,4];
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Abstract: Humans and non-human primates are vulnerable to age- and menopause-related decline in working memory, a cognitive function reliant on the energy-demanding recurrent excitation of layer III neurons within Area 46 of the dorsolateral prefrontal cortex (dlPFC). Here, we tested the hypothesis that the number and morphology (straight, curved, or donut) of mitochondria in dlPFC presynaptic boutons are altered with aging and menopause in the rhesus monkeys and that these metrics correlate with delayed response (DR) accuracy, a well-characterized measure of working memory. While bouton size was not significantly different across groups, aged postmenopausal monkeys had a higher percentage of boutons with mitochondria and a higher number of total and straight mitochondria per bouton compared to young and aged premenopausal monkeys. We next examined possible relationships between dlPFC mitochondria and the integrity of working memory, and found that the number of total and straight mitochondria per bouton showed significant positive correlations with DR accuracy.

In contrast, the frequency of boutons containing donut-shaped mitochondria, a morphological correlate of oxidative stress, exhibited a strong inverse correlation with DR accuracy. Lastly, we used a treatment known to enhance working memory to test whether mitochondrial morphology is regulated in relation to the treatment. Cyclic estradiol administration in aged ovariectomized monkeys, which significantly improved working memory, decreased the frequency of donut-shaped mitochondria. Together, our data suggest that hormone replacement therapy may enhance working memory, in part, by promoting mitochondrial health in the dlPFC.

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Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.19/KKK17

Topic: F.02. Animal Cognition and Behavior

Support: NIH/MIMH MH077298

Title: Regional-, laminar-, and target-specificity of prefrontal cortical pyramidal cell dendritic spine loss in response to dopamine denervation

Authors: H.-D. WANG[1], *A. Y. DEUTCH[2];
[1]Psychiatry, [2]Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: A number of post-mortem studies have reported decreased dendritic spine densities in the prefrontal cortex (PFC) in schizophrenia. Dopamine (DA) plays a key role in PFC function, and has been linked to certain cognitive deficits in schizophrenia. However, cognitive performance in schizophrenia is not globally affected, but restricted to certain domains, such as working memory. We examined dendritic spine loss in the cortex in response to DA loss. Disruption of the cortical DA innervation resulted in a decreased dendritic spine density in neurons of the medial PFC (areas 32) but not in the forepaw representation of the primary motor cortex. Subsequent analysis revealed that the loss of dendritic spines on PFC pyramidal cells (PCs) was restricted to layer V, and was not seen in layers II/III. We then examined dendritic spine loss in layer V PCs that project to a number of different target regions by retrogradely-labeling the PCs and then performing intracellular fills in these PFC PCs. We found that a very small minority (<10%) of layer V cells collateralized to innervate multiple subcortical targets such as the nucleus accumbens and ventral tegmental area. Dopamine depletion of the PFC resulted in a significant loss of dendritic spine density on layer V cortico-accumbens neurons. In

contrast, we did not observe a change in spine density in layer V cells projecting to the ventral tegmental area. Similarly, we did not uncover a loss of dendritic spines in PFC neurons innervating the amygdala, either from Layer V or Layers II/III. We also examined PFC PCs retrogradely labeled from the mediodorsal thalamus (MD). The projection from the PFC to the MD primarily originates in layer VI, with a smaller input from Layer V. Although no changes in dendritic spine density was seen in layer VI PFC cells projecting the MD, basilar and apical spine density was decreased by ~25% in layer V cortico-thalamic PCs. We are currently quantifying dopamine, serotonin, and glutamate receptor transcripts in the PFC neurons that innervate the various targets to determine the dopamine receptor subtype(s) involved in the dendritic remodeling seen after dopamine depletion of the PFC. These data suggest that specific circuits emanating from layer V of the PFC are dysfunctional in schizophrenia, and it may be possible to differentially relate dysfunction in one circuit (e.g., the circuit that emanates from the PFC to the MD and thalamofugal sites) may subserve specific symptoms (e.g., working memory deficits) in schizophrenia.

Disclosures: H. Wang: None. **A.Y. Deutch:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.20/KKK18

Topic: F.02. Animal Cognition and Behavior

Support: Australian Research Council DP110101200

Ramaciotti Foundation Equipment Grant

Title: Claustrum projections to the prefrontal cortex of the common marmoset: New insights regarding claustrum function?

Authors: *D. H. RESER[1], J. CHAN[1], K. BURMAN[1], K. WORTHY[1], X. PHAM[2], T. CHAPLIN[1], M. G. P. ROSA[1];
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Abstract: The function of the claustrum remains highly debated. Factors that complicate progress in addressing this question include the diffuse cortical connectivity, complex geometry, and relative surgical inaccessibility of this structure. Several hypotheses have been advanced suggesting a role for the claustrum in binding of polysensory information, and imaging studies suggest claustrum involvement in Alzheimer's disease, schizophrenia, and autism, but relatively

little detailed information about this structure is available. Here, using retrograde neuronal tracers, we mapped the claustrum projections to multiple histologically defined areas of the primate prefrontal cortex. Fluorescent tracers were injected into 18 sites in prefrontal cortex of 12 adult marmosets (Callithrix jacchus) under Alfaxan anaesthesia (8 mg/kg). After 1-3 weeks survival time, brains were extracted, postfixed, and cryoprotected, then sectioned (40 micron thickness) for histology and microscopy. All procedures were approved by the Monash University Animal Ethics Committee. Labelled cells were mapped and digitized using fluorescence microscopy and visualized using the CARET software package.
The distribution of labelled cells showed a marked rostral-caudal inhomogeneity, with projections to prefrontal areas involved in monitoring of internal states and executive functions (e.g. BA10, BA9, BA8B) located in the rostral claustrum, while areas involved in higher order and polymodal sensory processing (e.g. BA8aV) restricted to the caudal portion of the claustrum. Claustrum projections to the ventromedial prefrontal cortex and subgenual anterior cingulate cortex (BA32 and BA24) were tightly clustered in the rostro-medial claustrum. The pattern of claustrum projections to prefrontal cortex of the marmoset strongly suggests involvement of the rostral claustrum in the primate default mode network (DMN), which includes the prefrontal areas 8B, 10, 9, and 32, as well as the anterior cingulate region 24. Although the DMN has been widely studied in the context of normal function, as well as its role in the clinical conditions described above, we are not aware of previous reports of claustrum involvement in this network. Our data point to a need for more detailed study of the interaction between the claustrum and DMN, as well as other resting state networks.

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Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.21/KKK19

Topic: F.02. Animal Cognition and Behavior

Support: TUBITAK Grant 112S010

ESOGU-BAP Grant 201211C102

Title: Enhanced c-fos immunoreactivity in the prefrontal cortex of male rats following pre- and postnatal stress exposure

Authors: *E. ULUPINAR[1,3], E. SOZTUTAR[1], E. COLAK[2];
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Abstract:
Prefrontal cortex has critical centers related to the cognitive functions, memory and emotional behaviors. Development of this region continues from prenatal period to late adolescent ages. In this study, we exposed male Sprague-Dawley rats to early-life stress by keeping the dam immobile for 3 hours per day in the last week of gestation and separating the pups from their mothers for 3 hours daily, until weaning. According to anxiety levels, pups from different litters were categorized into two groups, as high and low anxious, at postnatal day 30. Elevated plus-maze test was repeated at postnatal day 60 to evaluate the emotional condition of the animals. Then, rats were subjected to the forced swim test immediately before their perfusion. The level of neuronal activation was analyzed by c-Fos immunohistochemistry and the total number of c-Fos expressing neurons in the medial prefrontal cortex (mPFC) was estimated by the optical fractionator method using Stereo-investigator software. Elevated plus maze test results indicated that early-life stress exposure increased anxiety-like behavior in stressed animals compared to the control group. Histological analysis showed that total number of c-Fos expressing neurons was significantly higher ($p<0.001$) in the mPFC of high anxious group than those of low anxious and control groups.These results showed that c-Fos activation in the mPFC display a positive correlation with the anxiety level of animals exposed to early-life stress.

Disclosures: E. Ulupinar: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); TUBITAK grant #: 112S010 and Scientific Research Commission of ESOGU project #:201211C102. **E. Soztutar:** None. **E. Colak:** None.

Poster

288. **Prefrontal and Striatal Systems: Anatomy and Function**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 288.22/KKK20

Topic: F.02. Animal Cognition and Behavior

Support: NSF-GRFP

Tartar Trust Fellowship

Medical Research Foundation of Oregon

P30NS069305-01

Title: Anatomical characterization of long-range thalamic projections in mouse

Authors: *B. J. HUNNICUTT[1,2], B. LONG[1,2], D. KUSEFOGLU[1,2], H. ZHONG[2,1], K. SVOBODA[3], T. MAO[2,1,3];
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Abstract: The thalamus is a central relay and integration site for sensory, cortical, and subcortical information as it transmitted into and within the brain. While thalamic projections have been studied for decades, several questions remain about the details of connectivity between the thalamus' roughly 40 cytoarchitecturally distinct subdivisions and their cortical targets. First, it is poorly understood whether thalamic axons are guided to their precise cortical targets via intrinsic molecular cues or if they arrive at their approximate topographic location and refine their positions based on activity. In addition, the layer specific axon distributions of thalamic projections is incompletely understood, primarily due to a lack of comprehensive, yet detailed, mapping techniques. Finally, there is virtually no anatomical data obtained from the widely used genetic model system, mouse. Here, we employed a systematic, dual-color, focal viral injection strategy to fluorescently visualize thalamic axons at high resolution as they project to multiple mouse cortical regions. Using this data set, we generated the first comprehensive map of thalamic projections to every subdivision of the frontal cortex. In addition, we demonstrate the power of our technique to uncover layer-specific thalamocortical projections, and to reliably localize the origins of previously unidentified projections. Understanding the detailed projection patterns of thalamic inputs to the cortex is crucial for deciphering the dynamic roles that the thalamus plays in cognition. Our results serve as a necessary resource for future studies investigating the functional roles of thalamocortical circuits.

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Poster

289. **Accumbens I**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 289.01/KKK21

Topic: F.03. Motivation and Emotion

Support: CONACYT Grant RTDR290649

Title: Enhanced D2-type receptor activity in the nucleus accumbens shell facilitates conditioned same-sex socio-sexual motivation in male rats: Behavioral and cellular evidence

Authors: *R. TRIANA-DEL RIO[1,2], J. MORIN[3], C. LOZANO-FLORES[3], V. RAMÍREZ-AMAYA[3], G. CORIA-AVILA[2], R. PAREDES-GUERRERO[3];
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Abstract: Dopamine activity in the nucleus accumbens (NAcc) plays a funamentel role in motivated behavior and instrumental learning. Additionally, activation of D2R is required for the establishment of partner preference. In the present longitudinal study we tested incentive sexual motivation and partner preference of male Wistar rats to sexual stimuli (femenine and masculine) in three different experimental stages: 1) before cannula implantation, 2) after cannula implantation in the nucleus accumbens shell, 3) after a conditioning period which consisted in infusion of 1 uL (.25 ug/uL) of quinpirole (a specific D2R agonist) in the NAcc and cohabitation in individual cages with another sexually experienced male which bore peppermint scent as a conditioned stimulus (conditioning occurred every 4 days, for a total of three trials). In these 3 stages males were tested for incentive sexual motivation and partner preference in a two-goal compartment chamber (peppermint scented male vs. sexually receptive female). The behavioral results revealed that in the first 2 stages, the experimental males visited more frequently the receptive female, but after the pharmacological treatment with quinpirole, the males displayed more time in close contact with the other male, increasing genital exploration and play solicitations towards him. Additionally, quinpirole treated males displayed less mounts, intromissions and ejaculations towards females. Four days after the preference test the subjects were exposed to the conditioned stimulus alone (peppermint odor) during 5 min, then were left undisturbed for 20 min in their home cages and finally exposed 5 min to a known but neutral stimulus (mango odor). Brains were immediately collected and quickly-frozen. Coronal brain sections were process for fluorescent in-situ hybridization for Arc mRNA, in order to identify the anatomical location of neurons that respond to the different odors. We are currently evaluating how same sex conditioning enhanced by dopamine D2R induces Arc transcription in several brain regions involved in information-processing of the relevant stimuli. Preliminary results support the idea that same-sex partner preference can result from associative conditioning and may depend, in part, on dopamine activity in the NAcc shell

Disclosures: R. Triana-Del Rio: None. **J. Morin:** None. **C. Lozano-Flores:** None. **V. Ramírez-Amaya:** None. **G. Coria-Avila:** None. **R. Paredes-Guerrero:** None.

Poster

289. **Accumbens I**

Location: Halls B-H

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Topic: F.03. Motivation and Emotion

Support: FAPESP 2011/07469-9

CNPq 478876/2011-4

CNPq 304537/2012-8

Title: Long-withdrawal from repeated nicotine did not change NPY and CRH-R1 gene expression in the amigdala

Authors: *C. S. PLANETA[1,2], T. F. GOTTSFRITZ[1], P. BIANCHI[2];
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Abstract: Dysregulations in the NPY-CRF systems have been associated to behavioral changes observed during nicotine abstinence in rats. The goal of the present study was to investigate whether repeated treatment with nicotine during adolescence could modify the expression of neuropeptide Y (NPY) and corticotropin-releasing factor receptor type 1 (CRF-R1) gene expression in the amygdale following long-term withdrawal. Male adolescent (postnatal day 28) Wistar rats received three daily injections of nicotine (1.0 mg/kg; s.c.) or saline for 10 days. Ten days (postnatal day 27) after the last nicotine (NIC) or saline (SAL) administration, animals were killed by rapid decapitation and the amigdala was isolated, frozen in liquid nitrogen and stored in -80 º C freezer for posterior analyses. The (NPY) and (CRF-R1) gene expression were measured by the reverse transcription-polymerase chain reaction (RT-PCR). The results showed that repeated treatment with nicotine during adolescence did not change NPY or CRF-R1 mRNA levels ten days after the withdrawal from nicotine, these data does not exclude the possibility that changes in NPY-CRF systems occurred in the early abstinence period and are no longer present 10 days after the interruption of nicotine treatment. We can also consider that nicotine - induced changes during early adolescence are transient and may not be detected in the late adolescence phase.

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Poster

289. **Accumbens I**

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Productos Medix 000652

Title: The weight-loss drug diethylpropion increases LFP's delta power and inhibits neuronal activity of the nucleus accumbens

Authors: *K. BALASUBRAMANIAN, JR[1], J. SOLORIO[1], A. VARGAS[2], A. LUNA[3], M. MORENO[1], I. PEREZ[1], R. GUTIERREZ[1];
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Abstract: Despite the wide use of diethypropion (DEP) as an appetite suppressant and as a short term weight-loss therapy for more than 50 years, there is a paucity of information about the neuronal mechanisms by which DEP produces its anorectic effect. The nucleus accumbens shell (NAcS) is a brain region associated with the regulation of reward, motivation and locomotor behaviors, and it is a part of the neuronal circuit that controls appetite. However, it is unknown if DEP can modulate NAcS activity. To address this issue, we first evaluated the effect of DEP on body weight-loss and locomotor activity and then we performed multielectrode recordings of local field potentials (LFPs) and single unit activity in the NAcS while behaving rats received an intragastric DEP administration. Our behavioral results found that both doses of DEP 20 and 40 mg/kg significantly decreased food intake and induced body weight-loss and hyperarousal state that may last up to 9 hours. We also observed a dose dependent impairment on locomotor activity and a prominent head weaving behavior (a stereotypic movement of the head) and this effect was stronger at high doses. Finally our electrophysiological results shown that intragastric DEP administration (20 mg/kg) evoked a significant increase in power at delta (1-4 Hz) and decrease in power at theta (5-11 Hz) bands in the NAc's LFPs. Regarding single spike activity; we found that DEP strongly inhibits NAcS single neuron activity and changed the spiking pattern of neurons from irregular single spike mode to rhythmic bursting pattern. Taking all together, we conclude that DEP produces a synchronous activity state (at delta - theta frequencies) in the NAcS, and we posit that this rhythmic activity plays a potential role on the anorectic and weight-loss effects of DEP. Our results also indicate that DEP can partially act by modulating this brain reward region.

Disclosures: K. Balasubramanian: None. **J. Solorio:** None. **A. Vargas:** None. **A. Luna:** None. **M. Moreno:** None. **I. Perez:** None. **R. Gutierrez:** None.

Poster

289. **Accumbens I**

Location: Halls B-H

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Salud2010-02-151001

ICYTDF-PICSA12-126

Productos Medix 000652

Title: The peripheral satiety signal induced by cholecystokinin modulates the activity of single neurons of the nucleus accumbens shell

Authors: *C. PEREZ DIAZ, JR, M. MORENO, R. GUTIERREZ;
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Abstract: Cholecystokinin (CCK) is a gut hormone that decreases food intake and delays gastric emptying. CCK is secreted postprandially from the I cells of the small intestine after intake of protein and fat rich foods and its anorectic action appears to be mediated through cholecystokinin receptor subtype 1 (CCK1R) located on peripheral vagal afferents. Despite that CCK do not cross the blood brain barrier, vagal activation of CCK1R can modulate the activity of several brain regions involve on feeding behavior. In the other hand, it has been shown that pharmacological inactivation of the nucleus accumbens shell (NAcS) promotes overeating, whereas its electrical stimulation stops feeding behavior, suggesting that the NAcS is a key brain region involved on the regulation of food intake. Moreover, in rodents, exogenous administration of CCK globally modulates neuronal activity of the NAcS measured by mediated manganese-enhanced magnetic resonance imaging. However, it is unknown if exogenous CCK can modulate NAcS activity at a single neuronal level, in freely moving animals. To answer this question, we performed multichannel recordings of the NAcS in freely moving rats while they received i.p. CCK injections (2 µg/kg) either alone or accompanied by devazepide, an antagonist with high affinity to CCK1R. In our preliminary electrophysiological results we found that peripheral CCK can modulate (either increase or decrease) the firing rate of NAcS single neurons and this modulation may last up to 10 to 15 min. Next, we behaviorally determined that 50 µg/kg of devazepide completely blocks both the decrease of food intake (Ensure -chocolate flavored drink) and the delay of gastric emptiness -induced by CCK. We conclude that single neurons

located in the NAcS are sensitive to the peripheral CCK satiety signal and we are currently testing whether devazepide can block the modulation of NAc activity induced by exogenous administration of CCK. These data suggest that the NAcS is part of the gut-brain axis of some peripheral satiety signals.

Disclosures: C. Perez Diaz: None. **M. Moreno:** None. **R. Gutierrez:** None.

Poster

289. **Accumbens I**

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Productos Medix 000652

Title: In the Thy1-ChR2 mice, optogenetic activation of nucleus accumbens' inputs is sufficient to stops feeding behavior

Authors: *L. E. PRADO RODRÍGUEZ, R. GUTIERREZ;
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Abstract: The nucleus accumbens shell (NAc) is a brain region part of the reward system that is involved in many brain functions such as coding reward, motivation and feeding behavior. In this regard, pharmacological inhibition of the NAc promotes overfeeding, whereas electrical stimulation inhibits feeding behavior, suggesting that a pause on the activity of this region can "gate" and thus allow the overconsumption of palatable foods. This hypothesis then proposes that opposite global activity of the NAc has a bidirectional control over feeding. Herein, we tested that hypothesis by using optogenetic global stimulation NAc's input stimulation in the B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J mice. Thy1-ChR2 mice express the microbial opsin ChR2 mainly on glutamatergic principal cells of layer V of cortex, and in many other brain regions that also project to the NAc, but it barely expresses ChR2 on NAc's neurons itself. We implanted a unilateral fiber optic on the NAc, in order to optogenetically activate all axons of the NAc that express ChR2 in this mice. Animals were trained on a lick task in which they had to lick three times a sipper fill with 10% sucrose solution. In the fourth lick cycle a blue LED was

turn on, during 1 sec duration, the sucrose solution was always available. In each trial, mice could receive either no-stimulation or a pulse of 4, 7, 14 or 21 Hz. We found that lower frequency stimulation (4 and 7 Hz), in 5 out of 6 mice, there was no effect on licking, but at high pulse frequencies (14 and 21) mice immediately stop licking the sucrose solution and this effect last during several seconds (>12 s). In fact, after LED stimulation at high frequencies animals were not able to emit the next lick. Further experiments showed that optogenetic NAcS' input activation stops sucrose intake at any moment during the licking cluster that is either in the very first lick or during the 4 or 16 lick cycle. We found that even a very short stimulation (80 ms; two pulses at 21 Hz) was enough to stop sucrose intake in this mice. We also tested if NAcS' input stimulation was self-rewarding; we found that 2 out of 6 mice were willing to lick four times an empty spout in order to receive self-stimulation demonstrating that global activation of the NAc is sometimes rewarding. We conclude that optogenetic NAcS' input stimulation is sufficient to stop feeding behavior.

Disclosures: L.E. Prado Rodríguez: None. **R. Gutierrez:** None.

Poster

289. **Accumbens I**

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Topic: F.03. Motivation and Emotion

Title: Different populations of nucleus accumbens neurons are associated with approach behavior and reward

Authors: *C. J. BROKER, D. WANG, S. IKEMOTO;
The Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Previous studies have shown that the dopaminergic system in the nucleus accumbens plays an essential role in invigorating approach behavior and inducing reward(s) associated with abused drugs. However, little is known about how individual accumbens neurons are responsible for encoding this reward and its goal-directed signals.
We examined unit activities of the nucleus accumbens in mice trained to self-stimulate with photo-stimulation that excited dopamine neurons in the ventral tegmental area (VTA). We injected a Cre-dependent adeno-associated virus encoding channelrhodopsin-2 into the VTA and implanted an optical fiber in the VTA and a bundle of 8 tetrodes (32 wires) in the accumbens shell of TH::Cre mice.
Mice quickly learned to vigorously lever-press for VTA optical stimulation. We found that many

accumbens neurons exhibited increased or decreased activities immediately after lever presses, triggered by optical stimulation of dopamine neurons. Moreover, these accumbens neurons also responded to non-contingent VTA optical stimulation in a similar manner. Thus, these neurons may be important for encoding dopamine neuron-mediated reward. We also recorded other accumbens neurons that exhibited firing changes before (~1 sec) lever presses, and these neurons were typically not responsive to VTA optical stimulation. Thus, these neurons may be involved in approach behaviors.
Together, our results suggest that different populations of nucleus accumbens neurons are associated with approach behaviors and reward.

Disclosures: C.J. Broker: None. **D. Wang:** None. **S. Ikemoto:** None.

Poster

289. **Accumbens I**

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Topic: F.03. Motivation and Emotion

Support: This work was supported by the Intramural Research Program of NIDA/NIH.

Title: Neural activity of nucleus accumbens induced by rewarding optogenetic stimulation of VTA dopamine neurons

Authors: *D. V. WANG, C. BROKER, S. IKEMOTO;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Scientific questions on brain reward systems were prompted by the discovery that animals learn to lever-press for electrical stimulation of certain brain areas, a phenomenon known as intracranial self-stimulation. Since then, numerous studies using anatomy, pharmacology and electrophysiology methods have established dopamine neurons in the ventral tegmental area (VTA) projecting to the nucleus accumbens (NAc) as a key reward substrate. Recent studies using optogenetics confirmed that animals learn to self-stimulate VTA dopamine neurons, suggesting that excitation of dopamine neurons is sufficient in inducing reward. The question remains is how excitation of VTA dopamine neurons influences downstream brain areas. We sought to identify firing patterns that encode ‘dopamine neuron-mediated reward’ in the NAc. We injected the AAV-ChR2 viruses and implanted optical fibers in the VTA area of TH::Cre mice, a procedure that allowed us to specifically activate dopamine neurons through optical stimulation; a bundle of 8 tetrodes (32 wires) was implanted in the NAc shell for neural activity recording. We found that VTA optical stimulation in freely-behaving mice evoked fast

excitatory local field potential (LFP) responses in the NAc, and the amplitudes of this LFP correlated well with the animal's self-stimulation rates. Consistent with the LFP activity, 35% of the recorded NAc neurons showed fast phasic excitations, suggesting an excitatory input to the NAc from VTA dopamine neurons. We also recorded neurons that showed phasic inhibitions (17%). To determine whether these firing pattern changes were mediated by dopamine, mice were systemically injected with the dopamine D1 receptor antagonist SCH 23390. Although the antagonist decreased majority basal firing in the NAc, it did not abolish optical stimulation-evoked neural responses, suggesting that transmitters other than dopamine were released by VTA dopamine neurons. In light of recent in vitro studies showing that dopamine neurons can also release glutamate and GABA to depolarize or hyperpolarize post-synaptic neurons, our above results could be explained by the co-realease of glutamate or GABA from dopamine neurons. Together, our results suggest that VTA dopamine neurons may employ multiple neural transmitters for inducing reward in vivo.

Disclosures: D.V. Wang: None. **C. Broker:** None. **S. Ikemoto:** None.

Poster

289. **Accumbens I**

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Topic: F.03. Motivation and Emotion

Support: NIDA IRP

Title: Rapid fluctuations in nucleus accumbens glutamate levels during motivated glucose drinking behavior

Authors: *S. E. MYAL, K. T. WAKABAYASHI, E. A. KIYATKIN;
BNRB, NIDA IRP, NIH, Baltimore, MD

Abstract: Glutamate (GLU) is the brain's major excitatory neurotransmitter. Though it is thought to be critically involved in the development and regulation of motivated behavior, the measurement of rapid fluctuations in extracellular GLU during behavior has been inaccessible until recently. To clarify this issue, we used enzyme-based GLU biosensors coupled with high-speed, fixed-potential amperometry to examine changes in extracellular GLU levels in the shell region of nucleus accumbens (NAc) during a simple task of motivated glucose drinking. For four days, non-food or -water-deprived male Long-Evans rats were moved to the test chamber and pre-trained to drink 5-ml of 10% glucose solution presented in a cup; two cup presentations were performed per day at 2h intervals. After pretraining, rats were implanted with a unilateral

cannula in the NAc for future insertion of GLU sensors. After a 5-day recovery period, rats were re-trained in an additional drinking session one day prior to GLU measurement. Electrochemical recordings using GLU sensors were conducted in 8-hour sessions, which included two drinking episodes at 2h intervals. Parallel recordings with GLU-null (enzyme-free) sensors were used to exclude contributions of non-specific electrochemical interferents. Our preliminary data suggest that NAc GLU levels slightly increase during cup presentation and movement toward the cup, followed by a rapid decrease during glucose consumption. After glucose is fully consumed, GLU levels increase again, correlating with searching behavior and grooming before falling to baseline when the rat returns to a resting state. When a water-filled cup is presented instead of glucose, the rat drinks much less and GLU levels are variably increased. Our data suggest that NAc GLU levels fluctuate biphasically during motivated behavior, with increases associated with seeking behavior and decreases during consumption of a palatable substance. Supported by NIDA-IRP.

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Poster

289. **Accumbens I**

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Topic: F.03. Motivation and Emotion

Title: The rewarding and aversive effects of nicotine are encoded by differential neuronal population activity dynamics in the nucleus accumbens shell

Authors: *S. R. LAVIOLETTE, N. SUN;
Univ. of Western Ontario, London, ON, Canada

Abstract: The mammalian mesolimbic pathway comprising the ventral tegmental area (VTA) and nucleus accumbens (NAc) has been identified as a critical neural system involved in processing both the rewarding and aversive stimulus properties of nicotine. Within the VTA, nicotine dose-dependently produces either rewarding or highly aversive behavioural effects, both of which are strongly modulated by mesolimbic DAergic transmission (Laviolette & van der Kooy, 2003; Tan et al., 2009). Indeed, transmission through dopamine (DA) receptor populations has been shown to functionally modulate these effects directly within the NAc. Nevertheless, the neuronal mechanisms within the NAc responsible for the bivalent motivational effects of nicotine are presently not known. Using an unbiased place conditioning procedure combined with in vivo neuronal electrophysiological array recordings, we examined the effects of intra-

VTA nicotine reward and aversion behaviours on specific neuronal sub-population activity patterns during nicotine conditioning, including acquisition, recall and extinction phases of nicotine-related associative memories. We report that sub-populations of fast-spiking interneurons (FSI) or medium spiny neurons (MSN) within the shell region of the NAc (NAshell) display differential activity patterns during the acquisition and extinction of rewarding vs. aversive conditioning doses of nicotine, directly within the VTA. Thus, while the rewarding effects of nicotine were associated with inhibition of FSI and activation of MSN neurons, the aversive effects of nicotine were associated with the opposite pattern of neuronal population activity. Similar to previous reports, pharmacological blockade of DA transmission with the broad-spectrum DA receptor antagonist alpha-flupenthixol, reversed the motivational properties of intra-VTA nicotine, switching nicotine aversion behaviours into rewarding conditioned effects. Remarkably, DA receptor blockade concomitantly switched differential intra-NAshell FSI and MSN neuronal population activity from an aversion pattern to a reward pattern, concomitant with the observed behavioural nicotine motivational switch. Furthermore, we report that alpha-flupenthixol, similar to other neuroleptic drugs, dramatically decreased striatal gamma band activity within the 50-80 Hz range. Rewarding doses of intra-VTA nicotine were associated with decreased striatal FSI-associated Gamma band 80 activity whereas aversive doses increased Gamma band 80 levels. Again, this effect was reversed following DA receptor blockade, concomitant with a switch from behavioural aversion, to reward.

Disclosures: S.R. Laviolette: None. **N. Sun:** None.

Poster

289. **Accumbens I**

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Topic: F.03. Motivation and Emotion

Support: FAPESP – Process Number: 2012/14723-1.

Title: Chronic testosterone treatment can cause neuroplasticities in the nucleus accumbens of adolescent rats

Authors: *S. A. ENGI[1], C. C. CRESTANI*[2], C. S. PLANETA[2];
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Abstract: Studies suggest that testosterone interacts with the brain reward system by affecting the endogenous opioid peptide and dopaminergic system. However, few studies investigated testosterone-induced changes in gene expression in the mesolimbic system. The aim of this study

was to investigate the central effects of the chronic testosterone treatment in adolescent rats. We used adolescent male Wistar rats (PND 28) that received a single daily injection of testosterone (10 mg/kg, s.c.) or vehicle during 10 days. Three days after the last testosterone administration animals were decapitated and had their brains removed to measure endogenous opioid peptide dynorphin and the dopaminergic receptor D1 in the nucleus accumbens. This study showed that 10-day testosterone treatment caused a significant increase in the prodynorphin mRNA expression and a significant decrease in the D1 receptor mRNA expression in the nucleus accumbens. Our results suggest that the chronic testosterone treatment can cause central neuroplasticies in the reward system.

Disclosures: S.A. Engi: None. **C.C. Crestani*:** None. **C.S. Planeta:** None.

Poster

289. **Accumbens I**

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Topic: F.03. Motivation and Emotion

Support: NIH/NIDA Grant ROIDA023641

Title: Blockade of AMPA receptors in the nucleus accumbens core interferes with the expression of Pavlovian conditioned approach

Authors: *M. VEGA VILLAR[1], R. I. CAAMAÑO TUBÍO[2], J. C. HORVITZ[2];
[1]Psychology Dept., The Grad. Center, CUNY, New York, NY; [2]Psychology Dept., City Col. of New York, CUNY, New York, NY

Abstract: A large research literature implicates nucleus accumbens (NAc) dopamine in reward-related learning and reward-directed behavior. Research on the role of NAc glutamatergic transmission in reward-related learning and behavior has focused primarily on glutamate NMDA receptors, and less so on AMPA receptors. The aim of this work was to examine the role of NAc AMPA receptors in the expression of a simple Pavlovian conditioned approach response.
Male Sprague-Dawley rats received seven daily drug-free Pavlovian conditioning sessions. Each session was comprised of nine CS/US trials, and each trial consisted of (a) a VT 110-seconds inter-trial interval; (b) a seven-second tone and (c) the delivery of a food pellet six seconds after tone onset. On days 8 and 9, rats received pre-session intra-NAc microinfusions of either an AMPA antagonist (NBQX; 1μg/0.5 μl/side) or vehicle. Finally, rats received two additional drug-free sessions on days 10 and 11.
The results indicate that AMPA receptor antagonist NBQX infused to the NAc core suppresses

the expression of an already-acquired Pavlovian appetitive response. The relative influence of AMPA receptors in the NAc core on the hedonic value of the reward, the initiation of a reward-directed response and the animal's overall motor capacity is discussed.

Disclosures: M. Vega Villar: None. **R.I. Caamaño Tubío:** None. **J.C. Horvitz:** None.

Poster

290. **Ethanol**

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Topic: F.03. Motivation and Emotion

Support: NIAAA # P60AA007611-26

Title: Electrophysiological responses within the prefrontal cortex to cue-induced binge ethanol intake

Authors: *D. N. LINSENBARDT, C. C. LAPISH;
Psychology, IUPUI, Indianapolis, IN

Abstract: A heightened reactivity to alcohol-associated cues is hypothesized to contribute to the compulsive drive to drink. As such, understanding the brain regions and neural processes that regulate cue-evoked alcohol seeking are critical for developing novel and effective treatment strategies for alcohol use/abuse disorders. Interestingly, neural activity within the prefrontal cortex (PFC), measured as changes in the BOLD response, is robustly altered after presentation of environmental stimuli associated with alcohol. While these data clearly implicate the PFC as necessary for integrating alcohol-paired cues, little is known regarding the changes in neural firing and oscillatory activity that underlie the altered hemodynamic response.
We used a novel model of cue-induced alcohol intake developed in our lab, dubbed "2-Way Drinking" (2WD) together with in vivo electrophysiology, to determine how neural activity in the PFC was altered during presentation of alcohol paired cues. Subjects were alcohol preferring 'P' rats and their non-genetically predisposed (heterogeneous) founding Wistar population. In the 2WD task, a light illuminated on either side of a rectangular operant box signaled the location and availability of 10% ethanol solution; animals were required to move to the illuminated side for 10s of alcohol access. Extinction of responding for ethanol was also evaluated using water in lieu of ethanol. Implanted electrodes attached to moveable microdrives were incrementally lowered through the PFC to maximize cell yield prior to electrophysiological recording and behavioral testing sessions.
In both rat populations there were increases in ethanol seeking behavior and total ethanol

consumed throughout 15 daily ethanol 2WD sessions, and decreases in these measures throughout 5 subsequent extinction sessions. However, the significant positive relationship between seeking and intake broke down during extinction sessions, but only in P rats, with increased seeking behavior relative to intake. There were a wide range of individual neuron responses to alcohol and alcohol-associated cues during 2WD sessions. Alterations in firing rate occurred in response to the light cue, the sound of the sipper extending and/or retracting, during fluid intake, and immediately following fluid intake.
These results provide additional support for the PFCs involvement in integrating information related to binge-alcohol intake and suggest that the 2WD model could be used to develop a more comprehensive understanding of how alcohol-related cues contribute to differences in the vulnerability of developing and maintaining alcohol use/abuse disorders.

Disclosures: D.N. Linsenbardt: None. **C.C. Lapish:** None.

Poster

290. **Ethanol**

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Topic: F.03. Motivation and Emotion

Support: NIH Grant U01-AA019970-NADIA

Title: Adolescent intermittent ethanol exposure increased risk taking and decreased dopaminergic and cholinergic activity in adulthood

Authors: *N. BOUTROS[1], S. SEMENOVA[1], W. LIU[2], F. T. CREWS[2], A. MARKOU[1]; [1]Psychiatry, UCSD Dept. of Psychiatry, La Jolla, CA; [2]Bowles Ctr. for Alcohol Studies, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Binge drinking is prevalent during the adolescent period, a time of extensive neurodevelopment. Adolescent binge alcohol exposure may have long-term effects on brain and behavior which persist into adulthood. The present study investigated whether adolescent intermittent ethanol (AIE) exposure would alter adult risk-taking behavior and prefrontal dopaminergic and forebrain cholinergic neuronal marker levels in male Wistar rats. Adolescent (PND 28-53) rats received 5 g/kg of 25% (v/v) ethanol three times a day in a 2 days on/ 2 days off exposure pattern. In adulthood, risk-taking behavior was assessed in the probability discounting task under baseline conditions and after acute ethanol challenges (0, 1, 2, 3 g/kg). Immunohistochemical analyses assessed levels of tyrosine hydroxylase (TH), a marker for dopamine, in the medial prefrontal cortex, and choline acetyltransferase (ChAT), a marker for

cholinergic neurons, in the basal forebrain, in rats during adulthood (PND 215). Under baseline conditions, when the large reward was delivered with high probability, all rats showed a preference for the large reward. When the large reward became unlikely, control rats demonstrated a preference for the smaller, guaranteed reward. In contrast, AIE-exposed rats continued to prefer the risky alternative, even when large rewards were very unlikely. Acute ethanol challenges had no effect on risky choice and group differences were maintained, with AIE-exposed rats exhibiting more risky behavior than control rats. TH and ChAT immunoreactivity levels were decreased in AIE-exposed rats compared to control rats. Interestingly, risk-taking behavior was negatively correlated with ChAT, implicating decreased forebrain cholinergic activity in risky behavior in subjects exposed to AIE. Though risk-taking was not correlated with TH levels, the significantly reduced levels of TH and ChAT suggest that adolescent alcohol exposure has enduring neural effects and that these neural effects may result in behavioral phenotypes such as increased risk taking. In humans, increased risk taking could lead to maladaptive or dangerous behaviors and could also increase the likelihood of developing an alcohol use disorder in adulthood.

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Poster

290. **Ethanol**

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Topic: F.03. Motivation and Emotion

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KO1 AA16849

Title: Ganaxolone reinstates ethanol seeking in an operant procedure in mice

Authors: *M. RAMAKER, M. M. FORD, D. A. FINN;
Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Allopregnanolone (ALLO), an endogenous neurosteroid with potent positive allosteric actions at $GABA_A$ receptors, may be increased in brain and plasma following ethanol exposure. These increases are thought to contribute to ethanol's anxiolytic, anticonvulsant, and hypnotic effects. ALLO dose-dependently alters ethanol intake and reinstates ethanol seeking in rodents, consistent the idea that GABAergic neurosteroids modulate the reinforcing effects of ethanol. Recent work has studied ganaxolone (GAN), a synthetic analog of ALLO, due to its longer half-life and promising safety profile exhibited in clinical trials for epilepsy. Because GAN altered ethanol intake in several self-administration procedures in rodents, the present study examined whether GAN would reinstate ethanol seeking in mice. Using a sucrose fading procedure, C57BL/6 male mice were trained to lever press for access to a 10% v/v ethanol solution (10E) on a fixed ratio 1 schedule. The fixed ratio was increased every 2-3 sessions, and mice were then transitioned onto a response requirement 8, where mice had 20 minutes to complete 8 lever presses; upon successful completion of the 8 "active" lever presses, a cue light turned on, levers retracted, and 10E was available for 30 minutes. Presses on an inactive lever had no scheduled consequence. Mice were maintained on this schedule for 6 weeks, where they maintained a stable intake of ~0.8 g/kg ethanol per session during the last 4 weeks. Mice then underwent 3 weeks of extinction training where presses on either the previously active or inactive lever had no scheduled consequence. When lever presses on the previously active lever were consistently below 25% of the previous criterion of 8, the effect of systemic GAN (0, 10, and 15 mg/kg) on the ability to reinstate lever pressing was tested in a within-subjects design. There was a significant effect of GAN to reinstate active lever presses [$F(2, 20) = 3.575$; $p = 0.47$]; both 10 mg/kg ($p = 0.025$) and 15 mg/kg GAN ($p = 0.008$) significantly increased presses on the active lever. There was also a trend of GAN to increase presses on the inactive lever, indicating there may also be some non-specific motor effects of GAN contributing to the observed effect. Overall, the effect of GAN to increase the appetitive drive to seek ethanol is consistent with published effects of ALLO treatment. The results of this study add to the growing body of literature that GABAergic neurosteroids alter both consummatory and appetitive processes involved in ethanol self-administration.

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Poster

290. **Ethanol**

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Title: Ethanol concentration-dependent effects of binge drinking in the presence of HIV-1 infection

Authors: S. SARKAR[1], *S. L. CHANG[2];
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Abstract: Binge drinking is a common form of acute alcohol abuse particularly in the young adult population. It has been correlated with neurocognitive deficits and is a high risk factor for developing behavioral problems that can lead to accidents, suicide and spread of sexually transmitted diseases (STD). Alcoholic beverages contain various concentrations of ethanol (EtOH). Young adults prefer to binge drink with high EtOH concentration beverages, which can result in increased risk-taking behavior and subsequently the spread of STDs including HIV-1. We hypothesized that there are EtOH concentration-dependent effects of binge drinking at both the molecular and behavioral levels, and these effects are enhanced during HIV-1 infection. In this study, young adult HIV-1 transgenic (HIV-1Tg) rats were binge-treated with solutions containing 0% (water), 20%, or 52% EtOH for 3 d, then expression of the HIV-1 viral gene, tat, was quantified in different areas of the brain, liver, and spleen. Tat expression increased in all three organs of the adult 52% EtOH-treated HIV-1Tg rats. In a subsequent study, blood EtOH concentration (BEC) as well as EtOH metabolism and neurotransmitter receptor gene expression were examined in young adult HIV-1Tg and F344 control rats administered 0% (water), 8%, or 52% EtOH for 3 d. BEC was higher in the 52% EtOH-treated HIV-1Tg rats than in the control rats. EtOH metabolism gene expression was increased in the liver of the 52% EtOH control group, but not in the HIV-1Tg rats. Neurotransmitter receptor gene expression was increased in the spleen of the 52% EtOH HIV-1Tg rats, but not in the control rats. To correlate the molecular changes with behavior, we examined the motor functions of the HIV-1Tg and control rats after 2, 24 and 72 hrs after binge exposure to 0% (water), 8%, or 52% EtOH for 3 d. Locomotor Activity was significantly reduced in the HIV-1Tg rats, but not the control rats, given 52% EtOH both 2 hrs and 24 hrs after final treatment, but not at 72 hrs. Our data indicate that there are EtOH concentration-dependent effects of binge alcohol drinking in the presence of HIV-1 infection at both molecular and behavior levels.

Disclosures: S. Sarkar: None. **S.L. Chang:** None.

Poster

290. **Ethanol**

Location: Halls B-H

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Topic: F.03. Motivation and Emotion

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NIAAA T32 AA007468

AHA 12GRNT12040366

OHSU Research Scholars Award

Title: Pre- and postsynaptic actions of alcohol at cerebellar granule cell extrasynaptic $GABA_A$ receptors

Authors: *J. KAPLAN, C. MOHR, D. J. ROSSI;
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Abstract: Cerebellar sensitivity to alcohol (EtOH) impairment is associated with increased risk for developing an alcohol use disorder, but it is unclear how differences in behavioral sensitivity are mediated at the cellular level. Previous reports in low-EtOH consuming Sprague-Dawley rats found that EtOH enhances tonic $GABA_A$ receptor ($GABA_AR$)-mediated inhibition of cerebellar granule cells (GCs) by increasing vesicular GABA release from Golgi cells, which increases the frequency of spontaneous inhibitory postsynaptic currents and the magnitude of tonic $GABA_AR$ currents. Since enhancement of GC tonic $GABA_AR$ currents powerfully dampens transmission through the cerebellar cortex, it likely contributes to behavioral impairment. We used voltage-clamp recording of GCs in cerebellar slices from high- and low-EtOH consuming C57BL/6J (B6) and DBA/2J (D2) mice, respectively, and tested whether the magnitude of EtOH-induced enhancement of tonic $GABA_AR$ currents differed across EtOH consumption phenotype, with the hypothesis that EtOH would be less efficacious at enhancing tonic $GABA_AR$ currents in the high-EtOH consuming B6 mouse strain. We found that while EtOH enhanced tonic $GABA_AR$ currents in low-EtOH consuming D2 mice (similar to GCs in Sprague-Dawley rats), it surprisingly suppressed the magnitude of the GC tonic $GABA_AR$ current in high-consuming B6 mice. In both mouse strains and Sprague-Dawley rats, the net response of GC tonic $GABA_AR$ currents to EtOH depended on the balance of two counteracting processes: 1) EtOH inhibition of nitric oxide (NO) production causing an increase in presynaptic vesicular GABA release, and 2) EtOH-induced inhibition of GC extrasynaptic $GABA_ARs$, dependent on the level of postsynaptic PKC activity. Our data indicate that genotypic variation in cerebellar nNOS expression and basal PKC activity levels determines the polarity and magnitude of EtOH modulation of GC tonic

$GABA_AR$ currents, which may play an important role in cerebellar-dependent EtOH-related behavioral phenotypes.

Disclosures: J. Kaplan: None. **C. Mohr:** None. **D.J. Rossi:** None.

Poster

290. **Ethanol**

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Klingenstien Fellowship Award Neuroscience

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Title: Imaging of temporal activation of nicotinic and dopaminergic receptor stimulation following nicotine challenge in non-human primates

Authors: *C. MARTINEZ-RUBIO[1,2], B. K. JENKINS[3], J.-K. CHOI[3], S. R. PATEL[2], E. N. ESKANDAR[2];
[1]Clarissa Martinez-Rubio, Boston, MA; [2]Neurosurg., Massachusetts Gen. Hosp., Boston, MA; [3]Radiology, Martinos Ctr. for Biomed. imaging, Charlestown, MA

Abstract: Nicotine is one of the most addictive and widely abused drugs. Neuronal nicotinic acetylcholine receptors (nAChRs) are selectively modulated by nicotine exposure and are highly represented in limbic circuitry. Different combinations potentially have distinct patterns of channel conductance, agonist sensitivity, and activation/deactivation kinetics. Chronic drug abuse induces down-regulation in response to excessive stimulation as an adaptative mechanism. Evidence suggests that nicotine causes rapid desensitization and that this loss of receptor function promotes an up-regulation to compensate the decreased signaling of inactive receptors. These changes cause high affinity to nicotine, correlated with nicotine addiction. Another common feature of addictive drugs is the increase of dopamine (DA) levels in the ventral striatum, specifically the nucleus accumbens (NAcc). We have collected four data sets with nicotine challenge (phMRI only). Three naïve monkeys were scanned using a home-made 8

channel receive helmet coil. Continuous images using the IRON technique with injection of iron oxide contrast agent that allows for determination of changes in cerebral blood volume (CBV) before and after injection of nicotine were collected while simultaneous measurements of heart rate, blood pressure and end tidal CO2 were made. High dose nicotine injection (0.5 mg/kg) led to a number of changes in CBV that had very different time courses in different brain regions. We performed functional connectivity analysis using the time course of the CBV changes as a reference. The thalamus, region with the highest density of α4β2 receptors, showed a strong increase in CBV, transient with a peak at 2.8min and a return to baseline by 15min. This time course was also noted in the VTA. The orbital frontal cortex and the NAcc showed biphasic CBV changes with a short negative CBV component (peak at 2.8min) that switched to being positive with a longer time course (peak at 15min). The putamen shows only a long positive CBV time course (peak at 15min) that slowly returns to baseline over 60min. This rapid CBV component looks very similar to the rapid desensitization shown by α4β2 receptors in electrophysiological studies in response to nicotine. In contrast, the time course in dopaminergic regions such as NAcc and putamen is much slower showing a slow rise, a long peak, and a long time return to baseline that is more consistent with the time courses of nicotine-evoked DA release measured using microdialysis. Currently we are collecting simultaneous PET/phMRI with raclopride displacement to assess the DA response and model its time course.

Disclosures: C. martinez-Rubio: None. **S.R. Patel:** None. **E.N. Eskandar:** None. **B.K. Jenkins:** None. **J. Choi:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: DA01749

CA143187

Title: Strain-dependent performance in nicotine-induced conditioned place preference and striatal pCREB expression

Authors: *L. A. ORTEGA MURILLO[1,2], E. YILDIRIM[2], M. ADOFF[2], R. POOLE[2], T. GOULD[2];
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Abstract: The addictive nature of nicotine remains a significant health problem for society. Neural circuits underlying addiction extensively overlap with those that support motivation processes, and there are individual differences in the probability to develop an addiction to nicotine. Therefore, understanding the mechanisms underlying individual differences to the preference to nicotine may provide fundamental insights in unraveling the neurobiology of nicotine addiction. In the present study, a biased nicotine-induced conditioned place preference (CPP) protocol was assessed in eight inbred mice strains. As regions of dorsal striatum contribute to nicotine-induced CPP, and pCREB modulates learning, we also assessed the effects of nicotine on striatal pCREB expression. Animals were individually tested according to the following protocol. First, there was a habituation phase. Second, a drug-free preference for the chambers was determined. Third, CPP training started and animals were injected with nicotine (0.35 mg/Kg) or saline (control). Two CPP tests were performed. The first test was performed following three conditioning sessions and the second test after seven conditioning sessions. Finally, brains were dissected at the end of behavioral training and dorsal striatum was isolated to analyze pCREB levels using Western blots. Nicotine produced strain-dependent effects on CPP performance. Strains differences in CPP ranged from preference to the nicotine-paired chamber, no apparent CPP, to aversion to the place paired with nicotine. For some strains, the nicotine-CPP effects were dependent on the timing of the test, suggesting strain differences also for the acquisition and maintenance of CPP performance. Significant CPP effects were correlated with pCREB levels in the striatum, but only for some strains. These findings suggest that strain differences related to nicotine-induced preference involve differential pCREB activation in the dorsal striatum.

Disclosures: L.A. Ortega Murillo: None. **E. Yildirim:** None. **M. Adoff:** None. **R. Poole:** None. **T. Gould:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

Location: Halls B-H

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Topic: F.03. Motivation and Emotion

Support: MH 58755

T32-GM007108

Title: Effects of orbitofrontal inactivation on dopamine cell activity during a delay-based decision task

Authors: Y. S. JO, *W. FOBBS, S. J. Y. MIZUMORI;
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Abstract: An immediate outcome is preferred to a delayed outcome because animals discount the value of a reward if it is preceded by a delay. A large body of evidence suggests that both the midbrain dopamine (DA) system and the orbitofrontal cortex (OFC) play a critical role in delay-based decision making by encoding the subjective value of delayed rewards. However, only a few studies have investigated the interaction between the OFC and midbrain DA systems during delay discounting. In the current study, we investigated whether OFC inactivation altered DA cells' activity in a delay discounting task. Six Long-Evans rats were trained to choose between chocolate milk rewards of two magnitudes (0.05 or 0.3 ml) that were presented in food cups on either side of an elevated T-maze. Wooden barriers were placed before each food cup in order to prevent the rats from consuming the rewards until after they waited the appropriate delay period: 3 sec for small reward and 10 sec for large reward. The animals were implanted with recording tetrodes in the ventral tegmental area (VTA) and injection cannula in the OFC. Putative DA cells in the VTA were recorded during daily behavioral sessions consisting of two blocks of 18 trials (10 forced-choice and 8 free-choice). Prior to the second block, saline or muscimol (GABAA agonist) was injected in the OFC. Behaviorally, OFC inactivation decreased the preference for the large, delayed reward, when compared to baseline performance in the first block. Neuronal data showed that DA cells initially responded to the acquisition of reward, but over training, these reward responses disappeared. Instead, DA cells exhibited phasic responses when the wooden barriers were removed after the delay periods. The response shift was consistent with DAergic prediction error signaling. OFC inactivation caused two major effects on DA cells: 1) phasic responses to delay termination were significantly decreased; and 2) DA cells exhibited stronger phasic responses to reward acquisition as if the reward was unexpected. Together, these results support the view that the OFC contributes important information that allows the VTA to recognize a reward as expected, despite delays.

Disclosures: Y.S. Jo: None. **W. Fobbs:** None. **S.J.Y. Mizumori:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

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Program#/Poster#: 291.02/KKK40

Topic: F.03. Motivation and Emotion

Support: MH58755

Title: Reward-sensitive neural responses in the periaqueductal gray during a spatial working memory task

Authors: *V. L. TRYON, E. FORMAN, S. J. Y. MIZUMORI;
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Abstract: It is well established that ventral tegmental area (VTA) dopamine neurons play an important role in reward processing, decision making, and action selection. However, the brain areas that drive the VTA dopamine neurons are not as well established. Recent anatomical work shows that the periaqueductal gray (PAG) sends excitatory and inhibitory projections to both GABAergic and dopaminergic VTA neurons (Omelchenko & Seasack, 2010). In fact, the PAG provides the third heaviest subcortical glutamatergic input to the VTA (Geisler et al., 2007). The role of these PAG projections to VTA reward processing has not been extensively investigated. Rather the PAG is better known for its role in the behavioral responses to fear, vocalizations, and descending pain modulation. Therefore, our initial investigation of this question revealed the PAG contributes to the ability to discriminate different reward magnitudes. Reversible PAG inactivation during performance on a spatial working memory radial maze task resulted in a decreased preference to choose arms previously associated with large rewards before arms associated with small rewards. PAG inactivation also decreased consumption of the reward itself. The current study investigated PAG neural responses to reward encounters as a different group of rats performed on the same task as that used in the inactivation study. Long Evans rats were trained to retrieve large and small rewards (four or one 45 mg sucrose pellets) that were consistently found in the same locations on a radial eight arm maze. Once rats reached asymptotic performance levels on the task, they were implanted unilaterally with a 6 tetrode array aimed at the PAG. On test days, PAG neural activity was recorded while each rat performed five baseline trials followed by five trials with one of the following manipulations: large- and small-reward placement switch, unexpected reward omission, or no manipulation (as a control for time and experience on the maze). Initial analysis reveals that in a subset of PAG neurons, there was phasic excitation at the onset of reward encounters. Previous work from our lab utilizing the same task (Puryear et al., 2010; Jo et al., in press) has revealed that VTA dopamine neurons preferentially fire to large versus small rewards, and are inhibited by reward omissions. Thus, we expect further analysis to reveal similar patterns in reward-sensitive PAG neurons. These results, taken together with the inactivation study and anatomical evidence, suggest that the PAG may in fact play a role in reward related processing.

Disclosures: V.L. Tryon: None. **S.J.Y. Mizumori:** None. **E. Forman:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 291.03/KKK41

Topic: F.03. Motivation and Emotion

Title: Separate groups of dopamine neurons project to caudate head and tail

Authors: *H. F. KIM[1], A. GHAZIZADEH[1], K. S. SALEEM[2], M. K. SMITH[1], O. HIKOSAKA[1];
[1]NIH, NEI, LSR, Bethesda, MD; [2]NIH, NIMH, Bethesda, MD

Abstract: Many neurons in the monkey caudate nucleus respond to visual objects and change their responses as the monkey learns their values. Importantly, there are regional differences within the caudate: neurons in the caudate head (CDh) learn object values quickly (in several trials), whereas neurons in the caudate tail (CDt) learn object values slowly (across days) (Kim and Hikosaka, SFN 2011 & 2012). What are the synaptic mechanisms underlying such different time courses of learning? It has been widely suggested that dopamine (DA) plays a critical role in value-based learning. We thus considered two hypotheses; 1) same dopamine neurons project to both CDh and CDt, but regulate them differentially, 2) different populations of dopamine neurons project to CDh and CDt, and thus regulate them differentially.
To test which hypothesis is correct, we injected two different retrograde tracers into CDh (Diamidino yellow, DY) and CDt (CTB-alexa555, CTB555) in the same monkey, and examined retrogradely labeled cells in DA neuron areas: substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). We found that neurons projecting to CDh and CDt were located in the ipsilateral SNc (not VTA), but were largely segregated. The CDh-projecting neurons were located relatively widely in the rostral-medial-ventral region of SNc. In contrast, CDt-projecting neurons were confined in the caudal-lateral-dorsal region of SNc. Notably, neurons projecting to both CDh and CDt (i.e., DY and CTB555 double-positive) were rare: 9 out of 1059 CDh-projecting neurons and 280 CDt-projecting neurons.
To examine whether the CDt-projecting neurons in SNc were DAergic. We double-labeled the sections with CTB555 and tyrosine hydroxylase (TH) antibodies. We found that most of the CTB555-positive cells were TH-positive, suggesting that the neurons in the caudal-lateral-dorsal SNc projecting to CDt are indeed DA neurons. We plan to do the same experiment for CDh.
Our data support the hypothesis that different populations of DA neurons modulate the synaptic plasticity of neurons in CDh and CDt with distinct time courses so that the monkey can adapt to both flexibly changing values and stably maintained values.

Disclosures: H.F. Kim: None. **A. Ghazizadeh:** None. **K.S. Saleem:** None. **M.K. Smith:** None. **O. Hikosaka:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

Location: Halls B-H

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Program#/Poster#: 291.04/KKK42

Topic: F.03. Motivation and Emotion

Title: Ventrolateral prefrontal cortical neurons encode the stable value and novelty of visual objects

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Abstract: Detection of object value or its novelty is crucial for directing attention and saccadic choice. Ventrolateral prefrontal cortex (vlPFC) is uniquely situated to influence object based decision making since it relays object identity information from inferotemporal cortex to frontal eye field and premotor structures which control behavioral outputs (Ungerleider et al 1989; Miyachi et al 2005, Gerbella et al 2011).Here we report that vlPFC neurons are strongly activated when visual objects are passively watched and can discriminate between them based on their reward history and familiarity. Two rhesus monkeys (Macaca mulatta) were trained on an object reward association task using more than 100 fractals over multiple sessions. Half of the fractals (good) were paired with big reward while the other half (bad) were paired with small reward. On separate days after initial training, animals passively viewed random presentations of good and bad fractals while fixating a central dot. Visually responsive neurons in vlPFC as a population fired significantly more to good objects compared to bad objects ($p<0.001$ ranksum). In fact, about 40% of neurons showed significant value discrimination even after accounting for object selectivity (two-way nested ANOVA value vs. object). Many of the task responsive neurons were also tested in a second experiment for differential responding to familiar vs. novel objects. Novel and familiar fractals with no reward history were intermixed and randomly presented to the animals while they maintained fixation in the passive viewing task. The population of task responsive neurons fired significantly more to passive viewing of novel objects compared to neutral familiar objects ($p<0.001$ ranksum). Interestingly, the strength of the novelty coding was significantly and positively correlated with the value coding (slope=0.4, $P<0.01$). In a separate task involving free viewing of objects, the monkeys made more saccades to and within good objects than bad objects as well as novel objects than familiar objects ($p<0.05$ t-tests). These results suggest that vlPFC may enable animals to attend and choose important (i.e., good or new) objects even in a passive condition based on long-term memory. Previous findings showed that substantia nigra pars reticulata (SNr) preferentially targets vlPFC through thalamus (Tanibuchi et al 2009). Importantly, SNr neurons have been recently found to strongly

encode stable values (Yasuda et al 2012). Whether novelty information reaches vlPFC via this nigrothalamocortical route or alternate routes remains to be examined.

Disclosures: A. Ghazizadeh: None. **O. Hikosaka:** None. **S. Hong:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: The intramural program of NEI/NIH

Title: Functional territories in the primate substantia nigra separately signaling flexible and stable values

Authors: *M. YASUDA, O. HIKOSAKA;
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Abstract: Some objects change their values flexibly depending on behavioral context, but other objects do not change their values for a long time. How do animals and humans adapt to the flexible and stable values? Recent studies from our lab suggest that different regions in the basal ganglia encode flexible and stable values of visual objects separately. Notably, neurons in the caudate head (CDh) encode flexible values, while neurons in the caudate tail (CDt) encode stable values (Kim and Hikosaka, SfN 2011, 2012). A major target of both the CDh and CDt is the substantia nigra pars reticulata (SNr). Previously, we found a cluster of neurons in SNr that exclusively encoded stable values of many objects and sent the signal to the superior colliculus (SC) (Yasuda et al., J Neurosci 2012). Where, then, is the flexible value information processed? We hypothesized that different territories in SNr encode flexible and stable values. To test this hypothesis we widely explored SNr beyond the cluster of stable value-coding neurons, and found a separate cluster of neurons that encoded flexible values. Whereas the stable value-coding neurons were localized in the caudal-lateral-dorsal part of SNr, the flexible value-coding neurons were distributed in the rostral-medial-ventral part of SNr. The response patterns of the flexible value-coding neurons were less stereotyped than those of the stable value-coding neurons. Specifically, the flexible value-coding neurons were sometimes influenced by stable values, whereas the stable value-coding neurons were hardly influenced by flexible values. Some of the flexible value-coding neurons (72%) were inhibited by high-valued objects and excited by low-valued objects, while others showed opposite responses. In contrast, a great majority of the stable value-coding neurons (93%) were inhibited by high-valued objects and excited by low-valued

objects. Importantly, many of both the stable value-coding neurons and the flexible value-coding neurons were antidromically activated from the ipsilateral SC. These results suggest that the stable value information and flexible value information are processed separately throughout the basal ganglia and may converge on SC neurons that control saccadic eye movements.

Disclosures: M. Yasuda: None. **O. Hikosaka:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

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Title: Negative feedback monitoring by lateral habenula and anterior cingulate cortex in monkey during a reversal learning task

Authors: *T. KAWAI[1,2,3,5], N. SATO[2], M. TAKADA[1], M. MATSUMOTO[3,4];
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Abstract: Neurons in both the lateral habenula (LHb) and the anterior cingulate cortex (ACC) respond to negative feedbacks. These signals have been proposed to play crucial roles in learning to avoid aversive events. It remains unclear, however, whether and how these signals cooperate for learning performance. To address this issue, we compared neuronal activities in the LHb and ACC in a monkey performing a reversal learning task. While the monkey was gazing a fixation point, two saccadic targets were presented on both the left and the right sides of the fixation point. The monkey was required to choose one of them. Saccade to one direction was followed by reward with 50% probability, while saccade to the other was not. After 20 to 30 trials, the rewarded direction was reversed without any instruction. The monkey learned to choose the rewarded direction by trial and error, and adaptively changed his choice based on the past reward history. Thus, the monkey chose the other direction with high probability as choosing one direction was repeatedly followed by no-reward. We recorded the activity of 38 LHb and 258 ACC neurons. Of these, 37 LHb and 117 ACC neurons showed a significant response to the feedback (reward or no-reward). Most of them (LHb, 37/37; ACC, 70/117) were more strongly

activated by no-reward. We found that the no-reward activation was influenced by past reward history only in the ACC. The no-reward activation in the ACC, but not in the LHb, was enhanced as no-reward trials were repeated, suggesting a parallel between the ACC signals and the monkey's choice behavior. The no-reward activation started later in the ACC than in the LHb. Furthermore, many neurons in the LHb and ACC responded to the fixation point. Only for the ACC neurons, this response was affected by the forthcoming decision as to whether the monkey might choose the left or right target. Notably, this "pre-choice" response was enhanced in the trials in which the monkey updated his choice from the previous trials. Our findings suggest that LHb neurons are useful to quickly detect negative feedback. On the other hand, ACC neurons convey history signals that would be relevant to learning to adaptively change behaviors.

Disclosures: T. Kawai: None. **N. Sato:** None. **M. Takada:** None. **M. Matsumoto:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

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Topic: F.03. Motivation and Emotion

Support: NIH Grant 31672A

CIHR postdoctoral award

Title: Co-release of glutamate and GABA in the lateral habenula

Authors: *S. SHABEL, C. D. PROULX, R. MALINOW;
UCSD, La Jolla, CA

Abstract: In the central nervous system excitation is generally balanced by inhibition. At synapses, this is primarily executed by local inhibitory interneurons mediating feedforward or feedback inhibition. However, some brain regions, such as the lateral habenula, lack inhibitory interneurons and therefore must use different mechanisms to regulate excitability. Here we've examined transmission between the entopeduncular nucleus (EP) and the lateral habenula (LHb), a pathway that may encode 'disappointment' and the presence of unpleasant stimuli. Recordings from LHb neurons in brain slices indicate that axons originating from the EP produce monosynaptic excitatory and inhibitory responses. Similar to feedforward inhibition in other neural circuits, monosynaptic inhibition suppressed neural activity evoked by high-frequency stimulation. Interestingly, EP neurons expressing the excitatory glutamate transporter VGlut2 or neurons expressing the GABA-producing enzyme GAD-67 each produce both glutamatergic and

GABA-ergic responses, indicating co-release of glutamate and GABA. Electrophysiological data suggest that glutamate and GABA are co-released from individual boutons and that co-release persists into adulthood. These findings demonstrate corelease of glutamate and GABA in the LHb, a process that may permit a local balancing of the net drive produced at specific input pathways and may be important for reward computations in the LHb.

Disclosures: S. Shabel: None. **C.D. Proulx:** None. **R. Malinow:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

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Topic: F.03. Motivation and Emotion

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NIMH IRP

Title: Effects of reward size and reward delay on ventral striatal tonically active neurons in monkeys engaged in an intertemporal choice task

Authors: *D. WEINTRAUB[1], J. WITTIG, Jr.[2], B. RICHMOND[2];
[1]Surgical Neurol. Br., NIH, Bethesda, MD; [2]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Introduction:
Tonically active neurons (TANs) of the striatum are believed to be cholinergic interneurons. Their responses are sensitive to aspects of reward-associated stimuli. To investigate the influence of reward valuation on ventral striatal neuronal responses, we are recording single unit neuronal activity during an intertemporal choice task in which the size of a reward and the time delay required to obtain it are varied in a crossed-design.
Methods and Results:
Two rhesus monkeys were trained to associate visual cues with various combinations of liquid reward amount and delay during a bar touch and release task. In each trial, a visual cue indicates the reward size:reward delay combination. The monkey's task is to release the bar when a red target turns green. The offer indicated by the cue can be accepted (wait for green) or skipped (release early). The monkeys accepted the trials in a predictable and reliable manner_large rewards with short waiting times were most preferred.
We recorded extracellular activity from 43 neurons with the slow regularly irregular firing

characteristic of TANs. Many of these neurons showed the burst-pause-burst pattern elicited by reward predicting stimuli seen in TANs by others in the past. We analyzed the neuronal firing rates following cue presentation and reward delivery according to the size and delay components using a two factor ANOVA. Following cue presentation, 18/43 (42%), 7/43 (16%), and 5/43 (12%) neurons showed significant main effects on firing rate of both reward size and delay, reward size alone, and reward delay alone, respectively. Following reward delivery, 4/43 (9%), 4/43 (9%), and 4/43 (9%) neurons showed a significant main effect on firing rate of both reward size and delay, reward size alone, and reward delay alone, respectively. Interaction effects of reward size and reward delay were seen following cue presentation and reward delivery in 16/43 (37%) and 6/43 (14%) neurons, respectively.
Conclusion: Both reward size and reward delay are encoded by tonically active neurons in the ventral striatum. In this intertemporal choice task, the effects of reward size and delay on neuronal firing are more frequent after initial cue presentation than after reward delivery. We find evidence for both main effects and interaction effects of the two components of reward value on the activity of these neurons.

Disclosures: D. Weintraub: None. **J. Wittig:** None. **B. Richmond:** None.

Poster

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Topic: F.03. Motivation and Emotion

Title: Subthalamic and pallidal neurons represent reward-related signals in a multistep task in a monkey

Authors: *H. IWAMURO, I. TRIGO DAMAS, J. A. OBESO;
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Abstract: One of the important roles of the basal ganglia is goal-directed and habitual control of behaviour. Several studies in rodents and primates have revealed that the dorsomedial and the dorsolateral striatum regulate goal-directed and habitual control, respectively. However, it is still unclear how the basal ganglia process neuronal signals in these two modes.
To investigate the signal processing of behavioural control in the basal ganglia, we recorded neuronal activity of the subthalamic nucleus (STN) and internal segment of the globus pallidus (GPi) in a monkey (Macaca fascicularis) performing a multistep task using a joystick and a

screen. The task consisted of three stages of variable difficulty: first, a reaching stage with a visible target, followed by a seeking period with an invisible target, which was considered more difficult than the previous one, and finally a simple button press for getting the reward.
A total of 70 STN and 123 GPi neurons were sampled. In the STN, 15 and 4 neurons showed phasic inhibitions and excitations of activity, respectively, after the target hits in both reaching and seeking stages. Since the physical movements at the moment of these neuronal modulations were different between the two stages, these neuronal responses were considered independent of movements. These neurons were found in the rostral part of the STN. On the other hand, in the GPi, 38 neurons which were distributed mainly in the rostro-medial part showed modulations of activity related to the target hit in both stages. According to the modulations around the target hit, they were categorized into four groups: 1) post-hit phasic inhibition type (n=9), 2) post-hit phasic excitation type (n=7), 3) post-hit long lasting excitation type (n=13), and 4) pre-hit predicting inhibition type (n=9). Each of them, except for type 4, showed the modulation in the same manner at both stages, although the seeking stage evoked stronger inhibition than the reaching stage in type 1 neurons as well as the STN neurons which showed inhibitions. In the type 4 neurons, the reaching stage inhibited their activity until the target hit, while the seeking stage hardly showed this pre-hit inhibition but evoked a phasic inhibition after the target hit. These results suggest that neurons in the rostral part of the STN and the rostro-medial part of the GPi process reward-related signals and their activity is affected by difficulty of accomplishment in goal-directed actions.

Disclosures: H. Iwamuro: None. **I. Trigo Damas:** None. **J.A. Obeso:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 291.10/KKK48

Topic: F.03. Motivation and Emotion

Support: HFSP, RGP0036/2009-C

JST, PRESTO

JSPS, 23115718

Title: Modulation of responses to the conditioned and unconditioned stimuli in the primate lateral hypothalamic area by an aversive option in Pavlovian conditioning

Authors: *A. NORITAKE, K. NAKAMURA;
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Abstract: The value of a reward may change when a punishment is included as one of possible outcomes. The same reward may be valued higher relative to a punishment, compared with when it is valued relative to 'no reward'. On the other hand, a reward may be devaluated under such risk of danger. In our previous study, we found that neurons in the primate lateral hypothalamic area (LHA) convey information mainly on reward probability, uncertainty, and predicted values, but not on punishment ones in a Pavlovian conditioning task. In the study, however, because rewards and punishments were used in separate blocks of trials, it remains unclear how such value coding is influenced when both positive and negative outcomes may occur. To examine this issue, we trained two monkeys (Macaca fascicularis) a bivalent (BIV) version, in addition to the appetitive (APP) and aversive (AVE) version of a Pavlovian conditioning task. In BIV block, either a liquid reward or an air-puff was used as US. In APP block, either a reward or a neutral tone;in AVE block, an air-puff or a tone was used as US. In each block, there were cued and uncued trials. On the cued trials, after a timing cue, a white dot, was presented at the center of the screen, one of the three visual conditioned stimuli (CSs) was presented. Each CS signaled 0, 50, or 100% probability of unconditioned stimuli (USs). After the CS disappeared, there was a 1.0 s delay followed by US. On the uncued trials, the US was delivered unpredictably. The cued and uncued trials were intermingled in a given block. Consistent with the previous report, among 244 task-related neurons, one-third showed graded CS responses depending on outcome probability (n=88) and/or graded US responses depending on outcome predictability (n=81) in APP block. We found that, by an inclusion of an aversive option, over half of such neurons lost graded modulation in CS (51/88) and US response (44/81) in BIV block. Conversely, some neurons which originally did not exhibit graded CS and US responses in APP block now gained an ability to grade CS and US responses in BIV block. These results suggest that activity of the neurons in the LHA is influenced by the different ranges of outcome values.

Disclosures: A. Noritake: None. **K. Nakamura:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

Location: Halls B-H

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Program#/Poster#: 291.11/KKK49

Topic: F.03. Motivation and Emotion

Support: DARPA REPAIR project N66001-10-C-2008

Title: Deciding to take action: Striatum activation during reaching to targets of varying reward value

Authors: *E. A. POHLMEYER, S. GENG, N. W. PRINS, J. C. SANCHEZ;
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Abstract: During activities of daily living, the environment presents many desirable and undesirable options to which an organism must respond. The striatum is believed to be part of a "limbic-motor" interface, providing integration of reward and motor information in order to contribute to the resulting behavioral actions. Better understanding of the information encoded by the striatum would provide new insights into how actions are influence by reward for developing theories of computational motor control. To do so, we recorded from a 16-channel microelectrode array in the striatum, targeting the nucleus accumbens (NAcc), of a marmoset monkey (*Callithrix jacchus*) while he performed two goal-directed reaching and grasping tasks. The first task focused on the monkey's decision of whether or not to reach for a single target item that was either desirable (one of two types of food items) or undesirable (a nonfood object). During each trial, the target item was presented at one of four spatial locations (orientated in a diamond pattern with the top and bottom locations providing a vertical target pair, and the left and right spots providing a horizontal target pair). The second task focused on the monkey's reach decision when two target items were presented simultaneously. All combinations of the three target items were used, with each trial's two target locations restricted to either the horizontal or vertical configuration. We found a large fraction of the recorded striatal neurons to be task correlated, exhibiting either excitatory or inhibitory responses (sometimes both), in a wide variety of activation patterns between the different task phases (e.g. decision making versus reach execution). Many cells responded during trial initiation, and/or the reveal of the target(s). Subpopulation of neurons appeared tuned to whether or not the monkey carried out a reaching movement (during either or both the decision and movement phases) with some neurons being explicitly tuned to whether movements were made to or withheld from targets at specific locations. When only one desirable target object was presented, we found little variation in the neural responses for the specific type of target. However, when the monkey had to decide between two target items, we observed cells whose tuning reflected both the specific type(s) of the potential targets as well as their locations. The variety of responses observed between the striatal neurons provides a rich pool of reward, decision making, and motor initiation information for computational motor controllers that mimic the brain's control processes.

Disclosures: E.A. Pohlmeyer: None. **S. Geng:** None. **N.W. Prins:** None. **J.C. Sanchez:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

Location: Halls B-H

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Program#/Poster#: 291.12/KKK50

Topic: F.03. Motivation and Emotion

Support: William J Flynn scholarship

Title: A single spiking neuronal model to account for the diverse spontaneous firing patterns of lateral habenula neurons

Authors: *A. LAVIALE[1], T. WEISS[2], R. W. VEH[3], M. T. MCGINNITY[4], L. MAGUIRE[4], K. WONG-LIN[4];
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Abstract: It is hypothesised that the lateral habenula (LHb) plays an important role in reinforcement learning and mood regulation. Experiments have shown that the LHb is excited by aversive events, is causally involved in avoidance learning, and its dysfunctions are implicated in clinical depression. The LHb receives inputs from the limbic system and the basal ganglia, and projects to the major dopaminergic and serotonergic nuclei. Previously, we have explored the morphological and electrophysiological properties of LHb neurons in rat brain slices. The cells were categorised into neurogliaform, spherical, fusiform, polymorphic and vertical cells. Observed spontaneous firing patterns fell into 4 categories: silent, tonic regular, tonic irregular and rhythmic bursting (SIL, TR, TIR and BST). An interesting finding is that, with the exception of neurogliaform cells, the membrane properties of the neurons are very similar, and morphology does not correlate with the firing pattern. Importantly, the injection of small currents can change the pattern from SIL to TR to BST. Our current hypothesis is that these neurons are functionally similar, with slight variability resulting in heterogeneous spiking behaviour. In this work we explore this variability using computational modelling.
We use the adaptive exponential integrate-and-fire neuronal model to replicate the firing patterns observed in LHb neurons. This model has been shown to be able to replicate a wide range of realistic spiking patterns, while remaining simple and very efficient. To identify parameters corresponding to the observed diversity of spiking patterns, a systematic computational approach is used. For each potential combination of parameter values, the model is tested and features such as frequency or number of spikes per burst are extracted and saved. Finally, a sliding window approach is used to identify a small region of the feature map containing the desired proportions of each of the observed pattern.
The model demonstrates all behaviours so far observed in the LHb with very similar neuronal properties. In addition, small input variations can result in the LHb neurons crossing between regions with different behaviours, resulting in large output variations. This hints at neurocomputational properties similar to a switching mechanism. In further work, this single cell

model will be expanded into a biologically inspired model of the LHb and its relations to other brain structures.
In summary, our work suggests that LHb neurons may operate in the vicinity of multiple behavioural regimes. Our computational search algorithm can also be extended to solve other computational neuroscience problems.

Disclosures: A. Laviale: None. **T. Weiss:** None. **R.W. Veh:** None. **M.T. McGinnity:** None. **L. Maguire:** None. **K. Wong-Lin:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

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Program#/Poster#: 291.13/KKK51

Topic: F.03. Motivation and Emotion

Support: NIMH-IRP

Title: Neural activity in primate striatum and amygdala during novelty seeking

Authors: *V. D. COSTA, V. L. TRAN, B. B. AVERBECK;
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Abstract: Novelty seeking refers to the tendency of humans and other animals to explore novel and unfamiliar stimuli and environments in pursuit of potential reward. Although there is evidence that novel stimuli activate striatum and amygdala for purposes of orienting, it remains unclear how these two areas facilitate decisions to explore novel options. To examine the role of these two regions in novelty seeking, we simultaneously recorded single-unit neural responses in ventral striatum and amygdala in two rhesus monkeys, as they played a version of a three armed bandit task. During the task, the monkeys learned to choose between three, probabilistically rewarded images. Periodically one of the three choices was replaced with a novel image the monkey had not yet associated with reward. We used a reinforcement learning model to derive the initial value the monkeys assigned to novel stimuli, in addition to ongoing value estimates for the two remaining familiar choices. Behavioral analyses indicated that the monkeys displayed an overall novelty preference, initially selecting the novel option more often than the best alternative option, and initially assigning novel options a higher value than familiar options. Single-unit electrophysiological recordings in ventral striatum and amygdala are ongoing and will be the main focus of discussion.

Disclosures: V.D. Costa: None. **V.L. Tran:** None. **B.B. Averbeck:** None.

Poster

291. **Neural Mechanisms for Reward-Based Decisions**

Location: Halls B-H

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Program#/Poster#: 291.14/KKK52

Topic: F.03. Motivation and Emotion

Support: Davis Foundation

Title: Encoding of reward-predictive cues in the ventral pallidum

Authors: *S. LARDEUX, S. M. NICOLA;
Psychiatry, Albert Einstein Col. of Med., BRONX, NY

Abstract: The nucleus acccumbens (NAc) has long been attributed a central role in linking motivation and action. Specifically, the NAc, under the influence of its dopaminergic innervation, facilitates cued reward-seeking when subjects must use "flexible approach" behavior to reach a fixed movement target from multiple possible starting locations (Nicola, J Neurosci. 2010). Many NAc neurons are excited by reward-predictive cues that trigger flexible approach; these excitations begin before onset of locomotion and predict the latency and speed of movement (McGinty et al., Neuron 2013). However, little is known about how these cue-evoked excitations in the NAc influence the activity of downstream structures in the basal ganglia to direct behavior.

To begin to answer this question, we recorded neuronal activity in the ventral pallidum, the major target of NAc projecting neurons, while rats performed a cued flexible approach task. In this discriminative stimulus (DS) task, rats had to make an operant response when the DS (an auditory stimulus) was presented but not when a non-predictive stimulus (NS) was presented; each stimulus was presented randomly on a 30 sec variable interval schedule. Animals were implanted with bilateral electrode arrays in the ventral pallidum. Most pallidal neurons we recorded were excited by the DS; we found three types of responses: a short activation, a longer activation or an inhibition. Most of the neurons activated by the DS responded also to the NS, but the activation was followed by an inhibition that was not present for the DS. Ongoing experiments characterize this firing in relation to cue-evoked flexible approach movement parameters, and future experiments will examine the effects of pharmacological manipulation of the NAc on pallidal cue-evoked activity.

Disclosures: S. Lardeux: None. **S.M. Nicola:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Topic: F.03. Motivation and Emotion

Support: NIH Grant MH083710 to DP

Title: Activity of basolateral amygdala neurons during risky foraging decisions

Authors: *A. AMIR, S.-C. LEE, M. M. HERZALLAH, D. PARE;
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Abstract: According to the evolutionary theory of emotions, fear is an inherited response tendency that promotes survival and reproductive success in the face of predatory risk. Previous work indicates that the amygdala plays a key role in the genesis of innate fear responses and the acquisition of new fear responses as a result of experience. However, there is little data on amygdala activity in naturalistic conditions, when animals must weigh the potential benefits of foraging against the increased risk of predation. To study this question, we recorded basolateral (BL) amygdala neurons in freely moving rats engaged in a foraging task. As in Choi and Kim (2010; PNAS), rats with restricted access to food were placed in an rectangular arena (8x2ft) divided in two compartments by a remote-controlled door: a dark nesting area (1ft) and a long brightly lit foraging area (7ft). Food pellets were placed in the foraging arena at various distances from the nest, in the absence or presence of a mechanical predator-like figure (Robogator). The Robogator was programmed to surge forward when the rat approached it. Even in the absence of Robogator, rats displayed cautious foraging behavior. When the door was opened, rats failed to approach the doorway in about 10% of trials. When they did, it took them 10 sec on average. Then, the rats waited by the doorway for up to 2 min before venturing into the foraging arena. After entering, they walked briskly, often along the wall, turned abruptly to take the food pellet and ran back to the nest to consume it. As the distance between the nest and food pellet was increased, so did the latencies to initiate foraging. When the Robogator was placed at the other end of the foraging arena, latencies increased further and the rats failed to initiate foraging in about 20% of trials. The vast majority of BL cells showed significant task-related activity. Upon approaching the doorway, the firing rates (FRs) of most presumed projection cells began decreasing, eventually reaching 20% of baseline by the time they retrieved the food pellet. In contrast, the FRs of presumed interneurons gradually increased, reaching 150-200% of baseline by the time the rats retrieved the food pellet. After food retrieval, FRs quickly returned to baseline in both cell types. Thus, even though predatory risk is enhanced during foraging, the activity of BL projection cells is suppressed, in part via the activation of BL interneurons. A

challenge for future studies will be to identify the origin of the top-down signals that suppress BL activity.

Disclosures: A. Amir: None. **S. Lee:** None. **M.M. Herzallah:** None. **D. Pare:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Support: NSF Grant 0748915 to BK

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NSF Graduate Research Fellowship to CCW

Title: Multiple neural circuits predict different types of financial risk taking

Authors: *C. C. WU, K. KATOVICH, B. KNUTSON;
Psychology, Stanford Univ., Stanford, CA

Abstract: To account for human financial risk taking, traditional finance models have focused on statistical moments of mean and variance. Higher order statistical moments such as skewness, however, can also influence choice. We have proposed that part of this influence may result from peoples' affective reactions to large but unlikely potential outcomes (e.g., wins in the case of lotteries, damage in the case of insurance). In this study, healthy human subjects (n=19) chose between risky gambles and certain gambles (i.e., 100% $0.00) for real money while being scanned with functional magnetic resonance imaging (FMRI). Gambles shared equal mean and variance, but varied in terms of skewness (i.e., positive skew: 25% +$5.25, 75% -$1.25, symmetric: 50% +$3.05 / 50% -$3.05; negative skew: 75% +$1.25, 25% -$5.25). Anticipatory neural activity (i.e., which occurred while subjects viewed gambles but before they selected their choice) was used to predict individuals' risky financial choices in both whole brain and volume of interest analyses. Whole brain analyses utilized GraphNet, a multivariate pattern analysis method optimized for FMRI, which implements a combination of regularization parameters designed to yield generalizable yet interpretable solutions (Grosenick et al., 2013), while volume of interest analyses used logistic regressions to regress localized brain activity on choice. Across all gamble types, the whole brain GraphNet solution predicted risky vs. certain choices at 65.8% (leave one subject out cross validation, $p<.001$). Visualization of features selected by the

GraphNet model revealed that positive features in the nucleus accumbens (NAcc) and negative features in the anterior insula predicted risky choice in general. Volume of interest analyses confirmed these associations over all gamble types, but additionally indicated that NAcc activity more robustly predicted risk seeking in positive skew trials, whereas anterior insula activity more robustly predicted risk avoidance in negative skew trials -- consistent with an anticipatory affect account (Wu et al., 2012). Together, these findings suggest that different neural circuits promote different kinds of financial risk seeking and imply that neurofinance models may account for choices that transcend the boundaries of traditional finance models.

Disclosures: C.C. Wu: None. **K. Katovich:** None. **B. Knutson:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Program#/Poster#: 292.03/KKK55

Topic: F.03. Motivation and Emotion

Title: Environmental enrichment affects novel object and location preference in adolescent rats

Authors: *D. E. COBB[1], R. E. EICHORST[2], D. C. KELLER[3], A. M. DOYLE[1], M. C. ZRULL[2]; [2]Psychology, [3]Biol., [1]Appalachian State Univ., Boone, NC

Abstract: Adolescence is a period of change that can include increased risk-taking. Interestingly, environmental enrichment (EE), which can enhance brain development, learning and memory, has mixed effects on exploratory behavior, which is an important component of novelty and sensation seeking. In this study, we examined how EE affected novel object and location preference in adolescent rats. Young Long-Evans rats (n=16) were exposed to EE in cages with ramps, platforms, inanimate objects, and other rats for 1.5 hr/day for two of every three days between postnatal days (PND) 34 and 64. Age-matched rats (n=16) were not enriched controls. Two-trial object and location preference testing occurred between PND 66 and 75 (15, 30, 60 minute and 24 hour delays). On Trial 2, a novel object (NOP task) or familiar object at a novel location (NLP task) was present in the test field. On PND 78, 8 EE and 8 control rats were sacrificed after a final, 2 hour EE session or 2 hours in the quiet and dark, and those brains were processed to visualize the c-fos protein. Neural activation in the amygdala was examined using microscopy and stereology. For 15 to 60 minute delays before Trial 2 of the NOP task, the proportion of novel object contact time increased for control rats (+43%, 0.54 to 0.77) and dropped for EE rats (-34%, 0.59 to 0.39). This trend reversed for the 24 hour delay ($p<.05$) when EE rats spent more time contacting the novel object (0.64 vs. 0.59). During Trial 2 of the NLP

task, EE rats showed stable contact with the object at the novel location for 15 through 60 minute delays and a decrease in attention to the novel location after 24 hours (-28%, 0.70 to 0.48). In contrast, control rats showed a consistent proportion of contact time with the newly located familiar object (0.60) across all delays ($p<.05$). Behavioral data suggest EE promotes adaptation to unfamiliar objects within a known environment over periods up to 60 minutes and to rearrangement of familiar objects in a known space at one day after initial exposure. Histology revealed differences in numbers of active neurons between EE and control rats. In basolateral amygdala of EE brains, there was a 19% reduction in active neurons relative to controls. However, rats with a final EE session showed 135% more fos-positive neurons than controls. While a history of EE appears to reduce baseline activity in amygdala, a single EE session can increase amygdala activity over that baseline. The data seem to suggest that in our rat model enrichment during adolescence may promote relatively short-term adaptation to the introduction of novelty into a familiar environment as well as longer-term adaptation to alteration or re-organization of items in a known environment.

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Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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NICHD

UCLA Center for Culture, Brain, and Development

SRCD Dissertation Grant

UC MEXUS Dissertation Grant

Title: Structural integrity of cortico-striatal white matter tracts and risk-taking correlates in adolescents

Authors: *D. GOLDENBERG[1], E. H. TELZER[2], A. J. FULIGNI[1], A. GALVAN[1]; [1]Psychology, UCLA, Los Angeles, CA; [2]Psychology, Univ. of Illinois at Urbana-Champaign, Champaign, IL

Abstract: In the United States, the majority of all deaths of youth aged 10-24 are from unnatural causes, due in large part to risky decision-making. Previous research suggests that risk-taking is associated with neural regions implicated in reward and cognitive control (i.e., striatum and prefrontal cortex), which undergo significant development during adolescence. The goal of this study was to use Diffusion Tensor Imaging (DTI) to examine the relationship between cortico-striatal white matter integrity and individual difference measures associated with risk-taking. Forty-eight adolescents (ages 14 -16 years, 56% female) were recruited from local schools. Endorsement of risk-taking correlates was assessed through well-validated measures, including the Zuckerman Sensation Seeking Scale (M=3.8, SD=1.5, range=0-10), Flinders Decision-Making Questionnaire (M=2.9, SD=.4, range=1-4), Benthin Risk-Perception Measure (M=2.0, SD=.7, range=1-7), and Conners Impulsivity Scale (M=1.9, SD=.5, range=1-4). Participants underwent magnetic resonance imaging (MRI) on a 3T Siemens scanner, during which a DTI sequence was collected to evaluate integrity of white matter microstructure.
Data were corrected for head motion, eddy current distortion, and signal loss. Each of the 64 direction files was then registered to the B0 image using a six-parameter registration in 2D to minimize eddy current distortions. Preliminary analyses suggest that individual differences in sensation seeking are associated with structural integrity of white matter connections between ventral striatum and medial prefrontal cortex. Planned analyses will implement Tract-Based Spatial Statistics (TBSS) to examine the relationship between fractional anisotropy (FA) values and self-endorsed risk-taking measures.
White matter architecture undergoes significant remodeling during adolescence. To our knowledge, no research has yet examined the link between white matter connectivity of cortico-striatal tracts and adolescent endorsement of risk-taking constructs. Risky behaviors at this time are likely associated with increased sensitivity to reward competing with a still-maturing ability to inhibit impulses. These analyses will elucidate the behavioral phenotypes (e.g. impulsiveness, sensation-seeking) associated with the structural integrity of connections between the multiple regions implicated in adolescent risk-taking. Research such as this will further inform our knowledge of networks involved in adolescent risk-taking, as the field moves towards a more integrated view of neural organization.

Disclosures: D. Goldenberg: None. **E.H. Telzer:** None. **A.J. Fuligni:** None. **A. Galvan:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Program#/Poster#: 292.05/KKK57

Topic: F.03. Motivation and Emotion

Title: Disadvantageous decision-making is related to increased motor impulsivity on a rodent gambling task

Authors: *M. M. BARRUS[1], J. G. HOSKING[1], F. D. ZEEB[2], M. TREMBLAY[1], L. COQUE[1], C. A. WINSTANLEY[1];
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Abstract: Impulsivity is understood as a range of behaviors that are lacking in foresight, inappropriately risky, prematurely expressed, and harmful to momentary and/or long-term success. Most component behaviors can be broadly classified as relating to choice or motor impulsivity, and the relationship between these forms is not well understood. Although high motor impulsivity is a key symptom of disorders like pathological gambling and addiction, in which decision-making on laboratory tasks is compromised, there have been no clear demonstrations that choice and motor impulsivity are related in the general population. Given that high levels of all forms of impulsivity co-occur in psychiatric disorders, the lack of concurrence between measures of impulsive choice and action in non-clinical subjects is potentially troubling, as this dissociation may mean that studying the regulation of impulsivity in normal populations cannot provide insight into the basis of impulse control disorders. The recurrent use of a rodent gambling task that measures both decision-making and motor impulsivity has provided our laboratory with data from a substantial number of animals (n = 221) which we evaluated to determine whether these two forms of disadvantageous behavior are related at the population level.
Our meta-analysis of these data revealed that motor impulsivity was positively correlated with poor decision-making under risk. Furthermore, when animals were divided into high-impulsive and low-impulsive groups based on their rate of impulsive action, clear differences were observed in choice preferences: more impulsive animals showed a stronger preference for the risky, less advantageous options. Highly impulsive rats were also slower to adopt an advantageous choice strategy and quicker to make a choice on individual trials. This work may represent the first demonstration of a clear relationship between choice and motor impulsivity in a non-clinical population.

Disclosures: M.M. Barrus: None. **J.G. Hosking:** None. **F.D. Zeeb:** None. **M. Tremblay:** None. **C.A. Winstanley:** None. **L. Coque:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Topic: F.03. Motivation and Emotion

Support: Virginia Tech Institute for Society, Culture, and Environment Summer Scholars Grant

Title: The interaction of reward sensitivity and executive function predicts substance use and neural signature of risk processing in adolescents

Authors: *N. LAUHARATANAHIRUN[1,2,3], J. KIM-SPOON[2], J. WALTERS[2], J. FARLEY[2], K. DEATER-DECKARD[2], W. K. BICKEL[1,2], P. CHIU[1,2,3], B. KING-CASAS[1];
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Abstract: Adolescence is a developmental period often characterized by increases in risky behaviors including substance use, reckless driving and risky sexual behaviors (Steinberg, 2007). Human neuroimaging and animal studies suggest that adolescents' proclivity toward risky decisions is likely related to differential development of subcortical and cortical regions (Casey et al., 2011; Steinberg, 2010). Immaturity in brain regions associated with cognitive control and enhanced sensitivity toward rewards combined with the lack of self-regulation ability to control impulses may explain adolescents' bias toward risky choices (Dahl, 2004; Luciana et al., 2012). The present study explores the extent to which the interaction between reward-seeking and cognitive control behavior are related to individual differences in behavioral and neural patterns of adolescents' risky decision-making.
To assess neural correlates associated with risky decision-making behavior, participants (N=24) engaged in a lottery choice task while their blood-oxygen-level-dependent response was monitored using functional magnetic resonance imaging. Adolescents were asked to make a series of decisions to either (i) accept the gamble at a cost, or (ii) reject the gamble. Gambles varied by risk level (low, medium, high) and expected value. In addition, reward sensitivity was measured using the Behavioral Activation System (BAS) and Behavioral Inhibition System (BIS) scales (Carver & White, 1994), while executive functioning was measured using standard neuropsychological tests (i.e., digit span, multi-source interference task, trail making, and Tower of Hanoi). Lastly, participants indicated their frequency of alcohol, cigarette, and marijuana use. Both behavioral and neural data indicate that adolescents with high reward sensitivity were likely to show higher levels of risky decision-making and substance use especially when paired with poor cognitive control. Specifically, adolescents with high BAS scores and low executive functioning scores exhibited greater bilateral insular activation relative to participants who had low BAS scores with high executive functioning. These data highlight how individual differences in reward sensitivity combined with executive functioning contribute to risky decision-making behaviors in adolescence.

Disclosures: N. Lauharatanahirun: None. **J. Kim-Spoon:** None. **J. Walters:** None. **J. Farley:** None. **K. Deater-Deckard:** None. **W.K. Bickel:** None. **P. Chiu:** None. **B. King-Casas:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 292.07/KKK59

Topic: F.03. Motivation and Emotion

Support: R01AA021121

T32AA07455

Title: Voluntary alcohol intake, specifically during adolescence, produces arrested behavioral development resulting in enduring maladaptive and suboptimal risk preference in adulthood

Authors: *A. G. SCHINDLER, H. H. TONG, S. WARLOW, H. GILL, J. J. CLARK;
Univ. of Washington, Seattle, WA

Abstract: Alcohol is the most commonly abused drug among adolescents and shows the highest liability of all abused drugs. In humans, adolescent alcohol use can predict later drug abuse potential, and can lead to negative alterations in decision-making functions. We have previously demonstrated that voluntary consumption of alcohol by adolescent rats (20 days, 10% ethanol or control gelatin prepared with 10% glucose polymers) results in increased maladaptive risk-taking behavior on a probability discounting task when later tested as adults. Here we extend these finding to show that adult rats that voluntarily consumed alcohol for 20 days did not differ from controls on the same probability-discounting task when subsequently tested, demonstrating that this effect is specific to the adolescent time window. Adolescence is characterized by maturation and remodeling of key brain regions implicated in reward and decision making. The malleable nature of the adolescent brain renders it uniquely vulnerable to environmental insults such as alcohol exposure. Thus, we hypothesize that adolescent alcohol intake results in arrested development of specific brain regions and neurotransmitter systems involved in decision making and leads to subsequent arrested behavioral development. In support of this hypothesis, we found that naive adolescent rats exhibited significant risk preference and that adult rats exposed to alcohol during adolescence mimicked this maladaptive behavior. Conversely, control adult rats and adult rats exposed to alcohol during adulthood were risk neutral, again demonstrating age specificity. With the use of fast scan cyclic voltammetry (FSCV) to measure subsecond dopamine release, we have shown that rats exposed to alcohol in adolescence demonstrate increased phasic dopamine transmission within the nucleus accumbens in response to risky, but not to safe, options in adulthood. These findings suggest that changes in striatal dopamine release, as a consequence of adolescent alcohol exposure, could bias choice by assigning greater

value to the risky option. Persistent changes in GABAergic function are seen following chronic alcohol exposure, which could potentially modulate DA release. In order to investigate this phenomenon further, flow cytometry was used to measure expression of GABAA receptor subunits on individual neurons of the ventral midbrain. The results of these experiments provide unique insight into the specificity of alcohol exposure on risk preference and the potential role that GABAergic modulation of dopamine neurons plays in this aberrant behavior.

Disclosures: A.G. Schindler: None. **H.H. Tong:** None. **S. Warlow:** None. **H. Gill:** None. **J.J. Clark:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 292.08/KKK60

Topic: F.03. Motivation and Emotion

Title: Risk-taking, fear and anxiety in Balb/c, C57BL/6J and CD-1 mice tested singly or in pairs

Authors: *A. ENNACEUR[1], R. M. ABUHAMDAH[2], P. L. CHAZOT[2];
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Abstract: The present study examined anxiety response in three strains of mice that were exposed singly or in pairs to a novel open space anxiety test. The group allocation is shown in the table below:

Singly **BA** (n=17) **C57** (n=14) CD1 (n=18)

Paired **BA**, n=8 vs. **BA**, n=8 **C57**, n=16 vs. **BA,** n=16 **CD1**, n=17 vs. **BA**, n=17

Groups and number of animals tested either singly or in pairs.
BA=Balb/c; C57=C57BL/6J; CD1= CD-1 (ICR)

When exposed to an unfamiliar platform elevated with downward steep slopes attached on two opposite sides, all three strains of mice spent a large amount of time in the areas adjacent to slopes than in any other parts of the platform. However, C57BL/6J and CD-1 crossed onto and explored the slopes while BALB/c mice did not take a risk, and remained the entire 12 min session on the platform. This suggests that exposure to the elevated platform induce fear and

anxiety which is elevated in BALB/c mice and reduced in C57BL/6J and CD-1 mice.
In the presence of a companion, from the same strain (BALB/c) or from different strains (C57BL/6J and CD-1), BALB/c mice did not cross onto the slopes, and they spent a large amount of time in the center of the platform compared to BALB/c singly tested and compared to C57BL/6J and CD-1 mice. In addition, not all paired C57BL/6J and CD-1 crossed onto the slopes. They were 7 out of 16 C57BL/6J mice (44%) and 12 out of 17 CD-1 mice (71%) which did not cross onto the slopes. This seems to indicate that a C57BL/6J and CD-1 mice were affected by the presence of BALB/c mice. The presence of a companion does not appear to mollify anxiety in high anxiety strain of mice. It appears to have a negative influence in the least anxious strains of mice.
The present study demonstrates that it is possible to assess anxiety and social interaction within a single test condition, and that our novel open space anxiety test can be useful in behavioral phenotyping and drug screening, particularly in animal models of social anxiety, schizophrenia and autism.

Disclosures: A. Ennaceur: None. **R.M. Abuhamdah:** None. **P.L. Chazot:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 292.09/KKK61

Topic: F.03. Motivation and Emotion

Support: Parkinson Society Canada

Canadian Institutes for Health Research

Title: Effects of the chronic D2/3 agonist ropinirole medication on rodent models of gambling-related decision-making

Authors: *C. A. WINSTANLEY, M. TREMBLAY, J. G. HOSKING;
Univ. British Columbia, Vancouver, BC, Canada

Abstract: Objective: The aim of this project was to investigate the effect of chronic administration of the dopamine D2/3 agonist ropinirole in two different models of gambling behaviour in rats. The more traditional drug treatment for Parkinson's Disease (PD), L-DOPA, has shown over time to produce debilitating side-effects such as dyskinesia. Selective dopamine agonists that act on the D2/3 receptors such as pramipexole and ropinirole have been used to treat the motor symptoms of PD, but these drugs may lead to a variety of impulse control

disorders (ICDs) including pathological gambling in some patients. Few studies have looked at the effect of chronic ropinirole on impulsive or risky behaviours.
Methods: One group of rats (n = 24) learned the rat gambling task (rGT)- a rodent analogue of the Iowa Gambling Task used clinically to assess decision-making under risk. Here, rats chose between four options, each associated with differing probabilities of reward and punishment. The second group of animals (n = 24) performed the Betting task- a paradigm which measures biases towards or away from risk. Here, rats chose between a guaranteed reward versus a 50:50 chance of double that reward or nothing. Behavior was assessed before and following implantation and removal of an osmotic mini-pump delivering either ropinirole at 5mg/kg/day or a saline solution for 28 days.
Results: Chronic administration of ropinirole increased premature responses on the rGT, but did not affect choice of the risky option. In the Betting task, chronic administration of ropinirole led to an increase in choice of the uncertain lever regardless of the baseline preference for the safe or uncertain lever characteristic of each rat.
Conclusions: This study suggests that chronic administration of the D2/3 agonist ropinirole produces an increase in preference for uncertainty which may explain why some PD patients treated with selective dopamine medication develop gambling and other risky and maladaptive behaviours. We intend to follow up these findings by determining the impact of chronic ropinirole in a dorsostriatal 6-hydroxydopamine (6-OHDA) lesion model of PD. These studies will help characterise the specific type of impairments experienced by PD patients taking dopamine agonist therapy and provide knowledge of the neurological mechanism underlying them.

Disclosures: C.A. Winstanley: None. **M. Tremblay:** None. **J.G. Hosking:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Program#/Poster#: 292.10/KKK62

Topic: F.03. Motivation and Emotion

Support: NIDA DA025875

Title: A home-cage operant paradigm for studying behavioral inhibition in the mouse

Authors: *R. P. FAUST, J. HINKLE, X. ZHUANG, J. A. BEELER;
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Abstract: With the emergence of sophisticated genetic tools that target specific neural circuits or signaling pathways, meaningful and sensitive behavioral paradigms are increasingly becoming the bottleneck in establishing a functional map of the nervous system. Operant conditioning paradigms are versatile paradigms that are commonly used to probe a variety of nervous system functions. However, they are typically labor intensive (weeks or months of daily sessions) and involve food or water deprivation that invariably alters the homeostatic system. Previously, we developed a semi-naturalistic and ethologically more relevant mouse home cage operant paradigm. Since mice live in this environment nearly undisturbed throughout the experiment and earn all their food via their operant responses (closed economy), food or water deprivation is not required. Moreover, it does not require much labor during the course of the experiment.
Here, we describe a behavioral inhibition paradigm adapting conventional Go/NoGo tasks to the home cage environment. Mice learn to inhibit operant responding for food reward during a discrete light cue, a 'NoGo' interval, and increase their responding when the cue is turned off, a 'Go' interval. The interval lengths are randomly distributed ranging between 10 and 120s and in continuous alternation. A NoGo interval does not terminate until the mouse has suppressed responding for the allotted period. The mice display pronounced learning curves in acquiring the task and reach asymptotic performance within 10-14 days. Unlike conventional Go/NoGo in mice, this paradigm does not require the use of aversive stimuli to motivate reliable and stable NoGo performance. Though less controlled than traditional, session-based paradigms, the homecage generates a much more extensive dataset that reflects entirely the mouse's self-regulated behavior in adapting to the task. This homecage version of Go/NoGo may offer an easier, higher throughput paradigm for assessing behavioral inhibition than current, conventional operant approaches, facilitating more rapid phenotyping of impulsivity in genetically altered mouse lines.

Disclosures: R.P. Faust: None. **J. Hinkle:** None. **X. Zhuang:** None. **J.A. Beeler:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Topic: F.03. Motivation and Emotion

Support: NIH Grant NS074014

NIG Grant DA033121

Daniel F. Ada L Rice Fdn.

Center for Compulsive Behavior and Addiction, Rush University

Title: A rapid method for evaluating risky decision-making in the rat using intracranial self-stimulation

Authors: *N. HOLTZ[1], S. TEDFORD[1], T. NAPIER[2];
[1]Dept. of Pharmacol., [2]Depts. of Pharmacol., Psychiatry, Rush Univ. Med. Ctr., Chicago, IL

Abstract: Impulsivity is a multifaceted behavioral feature common to a number of psychiatric disorders, including substance use disorders, ADHD, and impulse control disorders. One facet of impulsivity is increased risky decision-making. This is often investigated using probability discounting paradigms, in which subjects choose between small, certain reinforcers, and larger reinforcers delivered with varying uncertainty. Risky decision-making is characterized by a preference for the larger, uncertain (i.e., "riskier") reward. In studies with non human animals, food commonly serves as the reinforcer, often requiring food deprivation which can affect impulsivity itself. To circumvent this problem, we implemented intracranial self-stimulation (ICSS) of the medial forebrain bundle as the reinforcing stimulus for probabilistic discounting tasks (ICSS directly activates the neural circuitry involved with natural reinforcers) (Rokosik and Napier: J Neurosci Meth 198:260, 2011; Neuropsychopharm 37:1397, 2012). Our protocol consisted of nine phases that incrementally trained rats on increasing complex set of contingencies. The present study aimed to streamline this protocol. Following recovery from surgically implanting the bipolar stimulating electrodes, the rats were trained to associate the positively reinforcing electrical stimulation with a lever press using a forepaw (phase 1). Our prior studies had verified that 50Hz and 100Hz could serve as small and large reinforcers, respectively. Thus, rats were tested in FR-1 schedules of reinforcement at 50Hz and 100Hz (phase 2). Once rats distinguish between the two (phase 3), they were trained in the discounting task (phase 4). We present here several variations of the task attempted towards the development of a rapid method. Data collected thus far show that rats quickly acquire the task, omit fewer trials, and discount the larger reward at lower probabilities, suggesting that this abbreviated protocol retains the construct validity of the previous procedure, with the advantage of expedited data collection. This procedure may accelerate preclinical research examining the role of risky decision-making in psychiatric disorders, as well as a means to screen compounds for liability to enhance impulsivity.

Disclosures: N. Holtz: None. **S. Tedford:** None. **T. Napier:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Program#/Poster#: 292.12/KKK64

Topic: F.03. Motivation and Emotion

Title: Distinct types of uncertainty encoding in the primate septum

Authors: *I. E. MONOSOV[1], O. HIKOSAKA[2];
[1]NEI/NIH, Bethesda, MD; [2]Natl. Eye Inst., Bethesda, MD

Abstract: A key feature of decision-making and learning is sensitivity to uncertainty. Uncertainty about an outcome may bias subjects to approach and learn or avoid and ignore, to perform an action or to wait, to speed up or to slow down. However, the neuronal circuits mediating these uncertainty sensitivities are not well understood. In the primate septum, we found two groups of reward uncertainty-selective neurons (Type-1 and Type-2). We recorded neurons throughout the septum while monkeys participated in a Pavlovian procedure in which visual cues (conditioned stimuli; CSs) predicted rewards and punishments, with 100, 50, and 0% chance. In the most dorsal region of the septum, we found uncertainty neurons (Type-1) that were tonically activated during the presentation of the CS indicating uncertain reward-delivery (50% reward CS), but were not activated by the reward certain CSs (100% and 0% reward CSs) or reward deliveries. Type-1 neurons did not respond to any of the punishment CSs or punishment deliveries. Type-2 neurons were preferentially distributed in the ventral septum, including the diagonal band of Broca. They exhibited an increase in activity during the presentation of the reward uncertain CS (50% reward CS) and that activity continued to increase until the outcome (reward or no reward) was revealed. Unlike Type-1 neurons, Type-2 neurons also increased their activity during CSs that predicted 100% or 50% punishment, and were phasically activated by the delivery of punishment. Interestingly, Type-1 and Type-2 neurons exhibited different time scales of learning as the monkeys learned the meanings of novel 100, 50, and 0% CSs. Unlike Type-1 neurons which quickly acquired their representation of reward uncertainty while monkey learned the meanings of the visual cues, Type-2 neurons strongly responded to the novel stimuli and slowly learned to signal reward uncertainty, over the course of ~3 days. Our findings suggest that the septum contains multiple mechanisms for the processing and integration of uncertainty-related information and suggests a novel role for this brain region in behavior.

Disclosures: I.E. Monosov: None. **O. Hikosaka:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Program#/Poster#: 292.13/KKK65

Topic: F.03. Motivation and Emotion

Support: Grant-in-Aid for Scientific Research (KAKENHI)

Title: Financial risk attitude in Parkinson's disease patients

Authors: *S. KOBAYASHI[1], N. MATSUDA[1], Y. AKUTSU[2], Y. UGAWA[1];
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Abstract: Parkinson’s disease (PD) patients may exhibit impulse control disorders in relation to dopaminergic treatments. Previous studies examined PD patients using Iowa Gambling Task (IGT) and demonstrated their tendency of financial risk-seeking as a factor that biases reward-based decision-making. However, IGT requires multiple cognitive abilities including working memory and reward association learning, which may also be impaired in PD. To be free from such confounding, we developed Fukushima Gambling Task (FGT), in which reward magnitudes and probabilities of the choice options were explicitly indicated on the computer monitor. Subjects chose between safe (x yen with no risk) and risky (1000 yen at p%) options by pressing a button. The choice indifferent point X(p) was obtained for each p (5, 10, 25, 50, 75, and 90) by means of parameter estimation by sequential testing. In theory, risk-seeking behavior leads to greater X(p). The area under the indifference point curve was used as an indicator of risk attitude (FGT score). Cognitive functions were also assessed by Mini-Mental State Examination (MMSE), Frontal Assessment Battery (FAB), digit span, Raven Colored Progressive Matrices (RCPM), Rey-Osterrieth Complex Figure Test (ROCFT), Stroop Test, Wisconsin Card Sorting Test. IGT was also tested and risk attitude in IGT was scored by the difference in the number of choices between advantageous and disadvantageous card decks (IGT score). Learning during 50 IGT trials was evaluated by comparing the number of advantageous choices in the first and last 20 trials. Eight PD patients with MMSE score > 23 participated in the present study. FGT score indicated risk aversion in all subjects. In contrast, IGT score indicated risk-seeking behavior in three subjects and the effect of learning was absent in four subjects. FGT score showed negative correlations with the scores of MMSE, FAB, digit span, and ROCFT. Present study suggests that most PD patients are risk averse, and the risk-seeking behavior observed during IGT may be an overestimation due to other cognitive impairments. Interestingly, FGT demonstrated that PD patients with higher cognitive abilities chose a safe option at a price much smaller than the expected value of a risky option at all probability range, which is irrational and suboptimal for reward obtaining. It remains to be elucidated whether the risk aversion bias is a specific consequence of dopamine pathology in PD.

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Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Topic: F.03. Motivation and Emotion

Title: The dual orexin receptor antagonist almorexant decreases motor impulsivity in highly impulsive rats performing a rat Gambling Task

Authors: *L. F. COQUE[1], C. A. WINSTANLEY[1], M. A. STEINER[2];
[1]Psychology, Univ. of British Columbia, Vancouver, BC, Canada; [2]Actelion, Allschwil, Switzerland

Abstract: High impulsivity is a key symptom of psychiatric disorders such as bipolar disorder, substance abuse and attention deficit/hyperactivity disorder. The term impulsivity encompasses a range of behaviors which can be broadly divided into impulsive action and impulsive choice. In humans, impulsive decision-making under risk is often measured using the Iowa Gambling Task, in which subjects choose between 4 decks of cards to try and win money. In order to maximize their returns, subjects must avoid the high-risk options associated with large gains but also disproportionately large losses, and choose instead options that yield smaller incremental rewards but also smaller penalties. We developed a comparable paradigm for rats in which subjects have 30 minutes to earn sugar pellets by choosing between 4 different options. Just as in the IGT, the optimal strategy on this rat gambling task (rGT) is to favor options associated with smaller per-trial rewards but fewer time-out penalties, and avoid options associated with larger rewards but longer and more frequent penalties. A well-validated index of motor impulsivity is the degree of premature responding- the emission of a prepotent response before the signal is given. Such responses are commonly measured using the five-choice serial reaction time task, but are also recorded in the rGT.
Even though numerous types of impulse control deficit co-occur clinically, different forms of impulsivity may be underpinned by independent neurobiological mechanisms. Motor impulsivity in particular may be related to high levels of arousal, as stimulant drugs such as amphetamine and cocaine increase this form of impulsivity. The orexin system is strongly implicated in wakefulness and arousal, and orexin antagonists decrease reinstatement of drug-seeking in animal models of relapse in addiction. Interestingly, orexin antagonists can block the reinstatement induced by the anxiogenic drug yohimbine- a compound also known to increase motor impulsivity. We therefore tested whether the dual orexin receptor antagonist almorexant would decrease premature responding and risky decision-making on the rGT. Almorexant significantly and dose-dependently reduced premature responding, particularly in animals that made more of these impulsive responses at baseline. Such highly impulsive animals also showed

a significantly greater preference for the high-risk high-reward options. Although almorexant reduced this maladaptive choice pattern, this effect did not reach statistical significance. These results indicate that further investigation of orexin antagonists as a treatment for impulse control disorders may be warranted.

Disclosures: L.F. Coque: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Almorexant was donated by Actelion for this research. **C.A. Winstanley:** None. **M.A. Steiner:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Topic: F.03. Motivation and Emotion

Support: NIDA Grant 1T32DA031111-01

NIDA Grant DA027679

Title: Unpredictable chronic mild stress in adolescent rats causes hyperactivity, decreased anxiety, and risk-seeking tendencies

Authors: *E. K. KIRSCHMANN[1,2,3], E. C. DONNY[3,4], A. F. SVED[2,4], E. THIELS[1,2,3]; [1]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for Neurosci., [3]Ctr. for the Neural Basis of Cognition, [4]Psychology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Adolescence is a period of many neural, physiological, and behavioral changes, and of heightened vulnerability for the development of neuropsychiatric disorders. Unpredictable chronic mild stress (UCMS) is a widely-used model for inducing emotional instability, including depressive- and anxiety-like behaviors, in adult rodents. We wanted to study the effect of UCMS exposure during adolescence on emotional behavior.
We exposed adolescent rats (starting age: postnatal day 28-31; p28-31) to 5 weeks of UCMS (n = 16) or control conditions (pair-housed; n = 16), and assessed the effect of UCMS on a series of measures: body weight (weekly), sucrose preference (SPT; weekly), activity in a 2-hr open-field test (OFT; before UCMS and in week 5 of UCMS), performance on the elevated plus maze (EPM; in week 5 of UCMS), and novelty-suppressed feeding (NSF; in week 5 of UCMS). Rats were sacrificed at p62-65 for collection of trunk blood and harvesting of brains in preparation for measurement of plasma hormone levels and levels of proteins of interest in brain. Prior to sacrifice, some rats were treated with dexamethasone and exposed to an acute stressor (5-min

swim), to assess negative feedback functioning of the hypothalamic pituitary adrenal axis. UCMS attenuated growth and resulted in a phenotype characterized by hyperactivity and reduced anxiety. Weight gain from p28-p62 was significantly lower among UCMS- compared to control rats. Horizontal and vertical exploration, as well as time spent in the center of the OFT arena, were significantly higher in UCMS- compared to control rats. Although the total number of entries or time spent in the open arms of the EPM did not differ between groups, the number of rats that entered the open arms with a high frequency (1 standard deviation above the mean of control rats) was significantly higher among UCMS- than control rats. Similarly, although the latency to initiation of eating in the NSF test did not differ between groups, the latency to the first exploration of the food in the center of the arena tended to be shorter for UCMS- compared to control rats. Sucrose preference increased slightly over the course of the 5-week period in both groups, with no difference between them. Analyses of plasma hormone levels and proteins of interest in limbic and striatal brain regions are currently under way, as are studies to examine whether the effects of UCMS vary depending on the peri-weaning experience (weaned and shipped by supplier on p21 vs. shipped with dam on p18 and weaned on p21). Taken together, our present results suggest that UCMS during adolescence does not induce an anxious or anhedonic phenotype but instead yields a hyperactive, risk-taking phenotype.

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Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Topic: F.03. Motivation and Emotion

Support: DA027127 (JDR)

Hirschberg Undergraduate Research Grant Award (CGS)

Title: Role of nucleus accumbens in the modulation of individual risk attitudes

Authors: C. G. SINON[1], M. S. MCMURRAY[1], *J. D. ROITMAN[2];
[1]Psychology, Univ. of Illinois at Chicago, Chicago, IL; [2]Psyc, Univ. of Illinois at Chicago, CHICAGO, IL

Abstract: The pursuit of natural rewards often involves making choices with uncertain potential for payoff. Variations in the consistency of the reward delivery and the magnitude of reward received can bias an individual's attitude toward future risky decisions. The Nucleus Accumbens

(NAc) is a brain region in the basal ganglia which is important for encoding the affective value of rewarding stimuli. To determine the extent of the influence of NAc on modulation of risk attitudes, we used in vivo electrophysiology to record single neuron responses during a risky decision making task. Rats were trained to lever press for rewards that differed in magnitude and probability. One lever was designated as 'certain', with all presses rewarded with a smaller magnitude sugar pellet reward. Presses of other, 'risky', lever led to a larger magnitude sugar pellet reward on a probabilistic schedule, such that on a portion of trials reward was omitted. The probability of risky reward receipt was varied randomly from session to session across consistencies ranging from 12.5% payoff to 75% payoff, and the size of reward varied between 1 and 6 sucrose pellets. In this way, we could compare neural responses when the same objective outcome (e.g. risky reward of 4 sucrose pellets) may be evaluated differently (e.g. in a session with risk level of 25% vs. 50%, or compared with certain reward of 1 vs. 2 pellets). A total of 265 neurons were recorded over the course of 55 recording sessions. We measured neural activity in response to cues that predict lever availability, lever presses and reward outcomes across individual behavioral response strategies. We found that NAc neuronal activity was modulated at the time of cue onset, but did not show a strong relationship with subsequent choice. However, responses were modulated by session risk level and reward outcome.

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Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

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Topic: F.03. Motivation and Emotion

Support: NSERC Alexander Graham Bell Canada Graduate Scholarship

Title: Cholinergic modulation of decision making on the rat gambling task

Authors: *M. SILVEIRA[1], C. A. WINSTANLEY[1], M. SHOAIB[2];
[1]Univ. OF British Columbia, Vancouver, BC, Canada; [2]Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Pathological gambling is characterized by persistent, maladaptive gambling behaviour that causes significant functional impairment in personal and professional life. Our lab has previously developed an animal model of gambling behaviour called the rat gambling task (RGT), which is based on the clinically administered Iowa gambling task (IGT). In this task rats must choose between four lever options that are each associated with the delivery of a different

amount of reward, as well as different probabilities of receiving said reward. Like the IGT, rats quickly learn to select the advantageous options characterized by smaller rewards with lower penalties, and to avoid the large, higher penalty reward options. Previous work has shown that dopaminergic (d-amphetamine) and serotonergic (8-OH-DOPAT) drug agonists impair choice performance on the RGT. However, the role of acetylcholine (Ach) in producing the choice patterns observed has yet to be investigated. Cortical Ach mediates the detection, selection, and processing of stimuli and associations, all of which are processes likely involved in RGT performance. Sixteen male, Long-Evans rats were trained in the task and given subcutaneous injections of oxotremorine, a nonspecific muscarinic agonist, or scopolamine, a muscarinic receptor antagonist. Although oxotremorine (0.01 - 0.1 mg/kg) had no effect on choice, the highest dose of scopolamine (0.01-0.1 mg/kg) significantly impaired RGT performance. Interestingly, the impaired choice profile observed with scopolamine is similar to that previously reported with amphetamine. In line with these findings, we are currently assessing whether administration of oxotremorine can antagonize the performance deficit produced by amphetamine on the RGT. Overall these data show that muscarinic antagonists can impair decision-making performance, and suggest that the cholinergic system is an important mediator of gambling-like behaviour. Determining exactly how the cholinergic system interacts with dopaminergic and serotonergic systems to regulate decision-making on the RGT is an interesting and exciting avenue for future research.

Disclosures: M. Silveira: None. **C.A. Winstanley:** None. **M. Shoaib:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 292.18/KKK70

Topic: F.03. Motivation and Emotion

Support: Canadian Institutes of Health Research

Title: Dopaminergic and serotonergic modulation of loss aversion in a rat gambling paradigm

Authors: *W. K. ADAMS[1,2], P. J. COCKER[1], R. D. ROGERS[3], C. A. WINSTANLEY[1];
[1]Psychology, [2]UBC Inst. of Mental Hlth., Univ. of British Columbia, Vancouver, BC, Canada;
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Abstract: Loss aversion refers to the cognitive bias whereby subjects, when facing a choice between a probabilistic or guaranteed loss, consistently prefer to choose the risky option over accepting a definite loss. An extension of this phenomenon is seen in both recreational and

pathological gamblers who often gamble continuously to recover losses or “loss-chase” despite the existence of, sometimes severe, financial and emotional burdens. Understanding the neurotransmitter systems involved in mediating loss aversion may provide insight into the mechanisms by which it becomes maladaptive in a clinical context. We recently reported that dopamine D2, but not D1, receptor blockade and serotonin 5-HT1A receptor activation modulates loss-chasing behaviour in rats performing a novel gambling task in which sequences of unfavourable choice outcomes can be assessed. Here we explored the pharmacological modulation of behaviour in a modified version of this paradigm in which unfavourable choice options were presented in parallel rather than sequentially. 15 male Long-Evans rats were trained to make operant responses that produced either food rewards or, unpredictably, impending time-out periods in which rewards were unavailable. At these decision points, rats had to choose between waiting for these time-out periods to elapse before resuming responding for rewards ('quit' responses), or selecting risky options with a 50% chance of avoiding the time-outs altogether and a 50% chance of time-out periods twice as long as originally signalled ('chase' responses). Chasing behaviour was assessed following systemic administration of the following compounds: d-amphetamine; the D2 receptor agonist, quinpirole; the D1 receptor agonist, SKF 81297; the D2 receptor antagonist, eticlopride; the D1 receptor antagonist, SCH23390; and the 5-HT1A receptor agonist, 8-OH-DPAT. In this experiment using this newly-amended procedure, the percentage of chase responses was significantly increased by treatment with SCH23390 whereas eticlopride or 8-OH-DPAT administration produced no marked changes. While there was a tendency for reduced chase responding following amphetamine treatment this did not reach statistical significance. These data suggest that dopamine D1 receptors may be involved in mediating the expression of loss aversion in rats. Further research is required to elucidate the extent to which such gambling behaviour in rodents relates to the human condition.

Disclosures: W.K. Adams: None. **P.J. Cocker:** None. **R.D. Rogers:** None. **C.A. Winstanley:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 292.19/LLL1

Topic: F.03. Motivation and Emotion

Title: Contributions of tonically active neurons and uncertainty to striatal learning

Authors: *N. T. FRANKLIN, M. J. FRANK;
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Abstract: Computational reinforcement learning models of the basal ganglia often assume a fixed learning rate, making them suboptimal for flexibly adapting to non-stationarity in the environment. An optimal learner takes their own uncertainty into account to decide how much to update action values based on any given outcome. Here we consider how giant, cholinergic, tonically active neurons (TANs) may provide a mechanism by which to modulate learning as a function of expected and unexpected uncertainty. Constitutively active TANs were added to a previously published neural model of the basal ganglia by Frank, 2006. Effects of M4-muscarinic receptors activation were simulated through direct inhibition of direct and indirect pathway medium spiny neurons (MSNs). Effects of M1-muscarinic receptor activation were simulated through a persistent increase in leak channel conductance in the indirect pathway. A stereotypical burst-pause TAN firing pattern of varying duration was simulated during reinforcement feedback. By modulating MSN activity and learning, TANs improved probabilistic reversal learning but with a tradeoff: long TAN pauses result in better asymptotic performance whereas short TAN pauses facilitate speeded learning following reversal. This tradeoff arises from TAN modulation of the degree to which MSNs are active during feedback and thus eligible for learning. Longer pauses were also related to decreases in entropy among MSN unit activity during learning, suggesting a relationship between TAN unit activity and uncertainty. Dynamically adjusting TAN pause duration to scale linearly with entropy improved the model's performance across different levels of stochasticity in the reward schedule, both in terms of asymptotic error rate and learning speed following reversal. Simulations with TAN pause length held constant across training were typically able to perform well for a single stochastic environment, but suffered degraded performance across multiple levels of stochasticitiy. These findings suggest that TAN pause duration may be dynamically controlled by entropy of MSN activity signaling uncertainty in action values, promoting both stable and flexible learning regimes that are robust to changes in environmental uncertainty.

Disclosures: N.T. Franklin: None. **M.J. Frank:** None.

Poster

292. **Neural Mechanisms: Processing Uncertainty and Risk**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 292.20/LLL2

Topic: F.03. Motivation and Emotion

Support: 0858 OPGRC

Title: The insula cortex does not mediate reward expectancy in a rodent model of slot machine play

Authors: *P. J. COCKER[1], J. G. HOSKING[1], R. D. ROGERS[2], B. LE FOLL[3], C. A. WINSTANLEY[1];
[1]Psychology, Univ. of British Columbia, Vancouver, BC, Canada; [2]Psychiatry, Univ. of Oxford, Oxford, United Kingdom; [3]CAMH, Univ. of Toronto, Toronto, ON, Canada

Abstract: Gambling is a culturally and socially ubiquitous phenomenon that many people engage in without adverse effect. However, for a significant minority, gambling can become a maladaptive compulsion akin to drug or alcohol addiction. Cognitive theories of gambling have suggested subjective sensitivities to cognitive distortions or biases may underlie the development of pathological gambling. One of the most widely described cognitive distortions are near miss effects. Near misses are unsuccessful outcomes that are proximal to a win, such as matching two items on a pay-line and the third sliding by during slot machine play. Subjectively, near misses are experienced as aversive, but they have reliably been shown to galvanise further game play and may contribute to the purported virulence of slot machine play. Human imaging studies have shown increased insula cortex activation in response to near misses in healthy controls, although other investigations using pathological gamblers have yielded more equivocal results. We have developed a rodent slot machine task (rSMT) using standard operant chambers, wherein three flashing lights set to on or off following nose-poke responses. A win is signalled if all three lights set to on, whereas any other light pattern indicates a loss. Rats then choose between responding on a "collect" lever, which delivers sugar pellets on win trials but a 10 second time-out penalty on loss trials, or a "roll" lever which allows animals to start a new trial straight away. In a similar manner to humans, rats exhibit a near miss like effect, in that they show more erroneous collection responses on trials where 2-lights set to on i.e. that are structurally proximal to a win. Here, we demonstrate that the insula cortex is not critically involved in mediating slot machine play in rodents. Excitotoxic lesions to the granular insula failed to alter animals' ability to detect winning and losing outcomes or their reactivity to near miss trials. Likewise lesioned animals showed a broadly similar behavioural profile to control animals during extinction and reinstatement. In summary, it does not appear as though the insula cortex is critical for guiding reward expectancy during slot machine play.

Disclosures: P.J. Cocker: None. **J.G. Hosking:** None. **R.D. Rogers:** None. **B. Le Foll:** None. **C.A. Winstanley:** None.

Poster

293. **"Computation, Modeling, and Simulation IV"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 293.01/LLL3

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

Title: Evidence of motor skill specialization in wrist motion of skilled clinicians

Authors: *E. WADE[1], C. ZEISLER[1], J. CHEN[2], S. GHANIMANTI[2], J. WEI[2], C. TEMPLEMAN[2];
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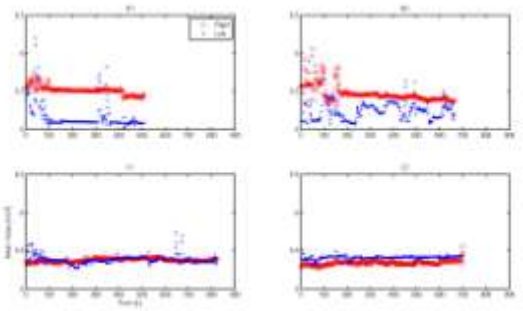
Abstract: Quantifying motor ability in skilled surgeons may provide insight into novel training techniques that enhance our understanding of motor skill acquisition and motor ability. Significant differences occur in kinematic measures of wrist motion of surgeons performing a suturing task [1]. Our present goal is to determine the time evolution of this motion during a laparoscopic skills lab.

Using a cross-sectional, within- and between-subjects design, four right hand dominant laparoscopic surgeons performed a suturing technique in an animal lab setting (Two 'experts' with fellowship training and two 'competent' without fellowship training).

Each expert (E1, E2) was paired with a competent surgeon (C1, C2) to perform intra-corporeal suturing. Each surgeon wore a set of 3-axis accelerometer wristbands on each upper extremity during the exercise. Supported by their correlate pair, each surgeon performed a series of two interrupted sutures twice. Comparative analyses for the periods of active intra-corporeal suturing were performed. Right and left hand performances were analyzed with paired t-tests for mean values of the vector sum of acceleration taken for 2s epochs of data.

E1 and E2 showed higher mean values in the right hand as compared to the left ($p<0.001$). C1 had no difference in means, while C2 showed higher means in the left hand ($p=0.19$, $p<0.001$, respectively, Fig 1). There was greater wrist motion in E1/E2 early in the procedure as compared to later, while C1/C2 maintain a constant level of activity. Further, E1/E2 have significantly higher amplitude in the right hand throughout the procedure, while C1 indicates no difference, and the difference in C2 is suppressed. Here we quantify specialization of expert surgeons as an increase use of the dominant hand. These results may indicate that during laparoscopic fellowship training, an increased focus on mechanisms to develop this specialization will influence the acquisition of expert skill.

[1] Chen J, et al. Wrist accelerometer analysis of competent versus expert laparoscopic surgeons. In submission, AAGL 2013.



Disclosures: E. Wade: None. **C. Zeisler:** None. **J. Chen:** None. **S. Ghanimanti:** None. **J. Wei:** None. **C. Templeman:** None.

Poster

293. **"Computation, Modeling, and Simulation IV"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 293.02/LLL4

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

Support: FAPESP #2011/21103-7 to RNW

FAPESP #2009/15802-0 to LAE

CNPq to AFK

Title: Influences of Ia feedback on low-frequency fluctuations of plantar flexion torque

Authors: *R. N. WATANABE, L. A. ELIAS, A. F. KOHN;
Dept. de Telecomunicações e Controle, Univ. of São Paulo, São Paulo, Brazil

Abstract: Several feedback loops are potentially involved while a subject performs a plantarflexion. One of these loops encompasses the proprioceptive feedback from muscle spindle Ia afferents and the motoneurons (MNs). In this work, the objective was to investigate the role of the Ia feedback loop on the low-frequency fluctuations of the plantar flexion torque during the performance of a position task (PT) and a force task (FT). Experiments were conducted on nine young healthy subjects and provided reference data for the computer simulation studies using a biologically-based large-scale neuromusculoskeletal model. The model consisted of: i) conductance-based spinal MNs arranged in three motor pools (for the Soleus and Gastrocnemii muscles); ii) muscle spindle model providing Ia afferent feedback; iii) Hill-type muscle models;

iv) second-order system to model the foot during PT; v) stochastic point processes to represent the activities of the descending neurons and of the afferents, which provide the randomness of the system. In the computer simulations the torque produced was equivalent to 20% of the maximum voluntary contraction, the same value adopted experimentally. For experimental data, torque power spectrum for the PT presented peaks at two different frequencies (3.40 ± 0.48 Hz and 6.24 ± 1.66 Hz) while for the FT only one peak was observed (1.67 ± 0.52 Hz). Simulation results reproduced the experimental torque power spectrum when the Ia proprioceptive feedback was active, but with a higher synaptic gain in the Ia-MN synapses for the PT. For the simulated PT two peaks were observed at 3.05 Hz and 6.01 Hz, respectively, whereas for the FT only one peak at 1.81 Hz was obtained. It is worth noting that for the FT the peak vanished when the Ia proprioceptive feedback was absent (Ia-MN synapses were turned off). Therefore, these results provide evidence that Ia afferent feedback may be responsible for the low-frequency fluctuations of plantar flexion torque found experimentally. It should be stressed that the muscle spindles during FT are active due to the low compliance of the muscle tendon. Further research will exploit the contributions of additional feedback loops to the torque spectrum and analyze the impact of these fluctuations on motor control.

Disclosures: R.N. Watanabe: None. **L.A. Elias:** None. **A.F. Kohn:** None.

Poster

293. **"Computation, Modeling, and Simulation IV"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 293.03/LLL5

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

Support: Canada Research Chair

CIHR

NSERC CREATE CAN-ACT

Title: A computational model of the kinematics of three-dimensional head-free gaze shifts

Authors: *M. DAEMI[1], D. CRAWFORD[2];
[1]Ctr. For Vision Res., Toronto, ON, Canada; [2]Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: We propose a kinematic model for the coordinated eye and head movements in order to make a gaze shift. Getting retinal error and initial eye and head orientations, this model

mathematically describes an experimentally-inspired sequence of rotations including saccadic eye movement, vestibule-ocular reflex (VOR) and head movement. The assumptions and constraints for solving the equations of the model are directly extracted from previous behavioral experiments in human and primates. Two main constraints are that the orientation of eye and head at the end of the gaze shift should respectively obey the Listing's law and the Fick law. This is a general model for the natural gaze shifts. We have defined two parameters in the model that control the amount of the head rotation such that for their very small values, the model is reduced to an experimentally-verified model for the head-fixed gaze shifts obeying all geometrical constraints for saccade. We also have defined another parameter in the model for controlling the amount of head rotation which contributes to gaze and the part of it which is cancelled out by VOR. This parameter subsequently determines the amount of VOR eye movement as well. These parameters are independent of the model structure and constraints so that they can be defined to be a function of any arbitrary variable like magnitude of gaze shift, initial eye and head positions and target saliency.
Based on this model and by arbitrarily setting the model parameters, the model accurately simulates the development of the spatial variables of the oculomotor system in different reference frames and in different situations. The model can predict where the gaze falls on a flat screen or where the image of the target falls on the retina during the gaze shift. The model can reproduce the behavioral differences between the symmetric and asymmetric gaze shifts. Having a 3-D model of gaze shift, we can use it to see how the torsional component of head and gaze are changing during gaze shift in different situations. Based on the mathematical relations in the model that have lead to the behavioral results, we can also think about the transformations that are required in the neural representation of such a model in order to implement a gaze shift.

Disclosures: M. Daemi: None. **D. Crawford:** None.

Poster

293. **"Computation, Modeling, and Simulation IV"**

Location: Halls B-H

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Program#/Poster#: 293.04/LLL6

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

Support: University of Minnesota Foundation

National Science Foundation grant No. 00006595

Title: A scalable analysis of network fluctuations and its application in magnetoencephalography

Authors: M. Y. MAHAN[1], *A. C. LEUTHOLD[2], A. P. GEORGOPOULOS[3];
[1]Biomed. Informatics and Computat. Biol., Univ. of Minnesota, Minneapolis, MN; [2]Dept Neurosci, Univ. Minnesota, MINNEAPOLIS, MN; [3]Dept Neurosci, Univ. Minnesota, Minneapolis, MN

Abstract: Networks of interacting elements exhibit global behavior that cannot be accounted for by the behavior of individual constituent elements but reflect interactions among those elements. One way to measure that global behavior is by obtaining an average of the absolute values of all possible pairwise correlations in the network. Although calculating all possible correlations is in itself a straightforward procedure, it is not scalable to the size of the network, and it quickly becomes intractable, as the number of pairwise operations (i.e. correlations) increases rapidly as the square of the number K of the network elements. Therefore, a scalable measure of global network behavior would be very desirable. Here we present such a measure, called "Departure from Network Equilibrium" (DNE). The cost for computing this measure increases linearly with K and, hence, avoids the combinatorial explosion above. Previously, we applied a similar analysis on a network of developing brain cultures with 60 elements (Christopoulos et al., J. Neural Eng. 9:046008, 2012), where we documented its positive relation to the average pairwise crosscorrelation. In the present study we develop the DNE analysis systematically and extensively using artificial network simulations to derive confidence intervals for DNE distributions and to apply this knowledge to a DNE analysis on brain activity recorded using 248 magnetoencephalographic (MEG) sensors from 169 healthy human subjects (age 31-97 y). We found that the standard deviation of the time-varying DNE decreases as a power function of K network elements, independently of time series length N. An investigation of the interactions between MEG-derived DNE networks showed a strong negative interaction between the left and right hemispheres, which decreased significantly with age, such that it became less negative and tended to a plateau toward zero in advanced old age. By contrast, there was a strong positive interaction within each hemisphere, which became less positive with age in the left but not the right hemisphere. These findings document the promising utility of the DNE approach for scalable analyses of networks.

Disclosures: M.Y. Mahan: None. **A.C. Leuthold:** None. **A.P. Georgopoulos:** None.

Poster

293. **"Computation, Modeling, and Simulation IV"**

Location: Halls B-H

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Program#/Poster#: 293.05/LLL7

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

Support: PAI UdeSA

CNPq (grants 480108/2012-9 and 478537/2012-3),

FAPERJ (grants E-26/111.655/2012 and E-26/110.526/2012).

FAPESP (grant 2011/51350-6),

Title: EEG functional networks in motor processing during visual stimuli

Authors: *D. E. FRAIMAN[1,2], G. SAUNIER[3,4], T. LEMOS[4], E. MARTINS[4], C. VARGAS[4]; [1]Matematica y Ciencias, Univ. San Andres, Victoria, Buenos Aires, Argentina; [2]CONICET, Buenos Aires, Argentina; [3]Inst. de Ciencias Biológicas, Univ. Federal do Pará, Belem, Brazil; [4]Inst. de Biofísica Carlos Chagas Filho, Univ. Federal de Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: We herein address the time evolution of brain functional networks computed from electroencephalographic activity driven by visual stimuli. We describe how these functional network signatures change in fast scale when confronted to point light display stimuli depicting biological (BM) as opposed to scrambled motion (SM) in two different conditions. Whereas global network measures (average path length, average clustering coefficient and average betweenness) computed as a function of time did not discriminate BM from SM, local node properties did so. Contrasting BM vs. SM network local measures we found higher degree and betweenness values in the left frontal (F7) electrode, as well as higher clustering coefficient in the right occipital (O2) electrode for the SM condition. Conversely, for the BM condition we found higher degree values in central parietal (Pz) and clustering coefficient in the left parietal (P3) electrodes. These results add on the understanding of cortical networks enrolled in the coding of biological motion. Thus, functional network approach is of mathematical election for studying brain function in the time scale of cognitive processing, allowing a new level of understanding of the complex phenomena associated with brain function.

Disclosures: D.E. Fraiman: None. **G. Saunier:** None. **T. Lemos:** None. **E. Martins:** None. **C. Vargas:** None.

Poster

293. **"Computation, Modeling, and Simulation IV"**

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Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 293.06/LLL8

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

Support: CSC

DFG Grant #IGK1247

Title: A bio-inspired CPG model for hierarchical control of limbless locomotion

Authors: *J. ZHANG, G. LI;
FB Informatik, TAMS, Univ. of Hamburg, Hamburg, Germany

Abstract: This work presents a bio-inspired CPG model for locomotion control of limbless robots, concentrating specifically on the study of a hierarchical control architecture as steps toward developing limbless robots capable of 3D locomotion, fast reflex response and sophisticated response to environmental stimuli. The key issue for developing such a hierarchical control architecture is how to design a CPG based controller that not only can generate various gaits, but also provide a solution for realizing reflex mechanism as well as integrating sensory feedback.

To this end, we first design a CPG model inspired by the neuronal circuit diagram in the spinal cord of swimming lampreys. A set of interneurons described with sigmoid function and leaky integrators is incorporated into the design of the neural oscillator for rhythmic signal generation. Furthermore, according the connection between neural oscillators, a chained type and a cyclic type of CPG circuits are developed for generating traveling waves between oscillators. Through numerical simulations, the control parameters over relevant characteristics of the two types of CPG circuits are studied in detail.

We further design the proposed CPG model for limbless gaits implementation. Considering the configuration of limbless robots with pitch modules and yaw modules connected alternatively, two CPG circuits are applied to the pitch grouped modules and the yaw grouped modules, respectively. Both the necessary conditions for cooperation between the two CPG circuits and the control parameters for fast limbless locomotion are investigated. Four types of limbless gaits, i.e. side winding, rolling, turning and flapping have been realized. Results of simulations and experiments show the effectiveness of the proposed CPG circuits in generating limbless locomotion.

We also present the concept of sensory neuron and reflex arc. Since the proposed CPG model is derived from neural circuit in lampreys´ spinal cord and the existence of sensory neurons in lampreys have been proven, it is simple and natural to add sensory neurons into the proposed CPG model at the neuronal level. Based on the design of the sensory neurons, a reflex mechanism taking advantage of reflex arcs forms short pathways to bridge external stimuli and the CPG model. Thus fast response can be computed when the external stimuli is afferent to the CPG model. A ball hitting and a corridor passing experiments with multi-joint limbless robots confirm the feasibility of the reflex mechanism.

Disclosures: J. Zhang: None. **G. Li:** None.

Poster

293. **"Computation, Modeling, and Simulation IV"**

Location: Halls B-H

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Program#/Poster#: 293.07/LLL9

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

Support: FAPESP Grant 2009/15802-0

FAPESP Grant 2011/21103-7

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Title: Large-scale neuromusculoskeletal model used to investigate neurophysiological mechanisms behind upright stance control

Authors: *L. A. ELIAS, R. N. WATANABE, A. F. KOHN;
BME Lab, PTC, EPUSP, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Several models have been employed to study the basic mechanisms underlying postural control in humans. Nevertheless, the vast majority of these models are based on a control engineering framework, so that the nervous system is approximated by a proportional-integrative-derivative (PID) linear model. While this may be a valid assumption, it fails in providing a close link between theory and physiology. In this study, our aim was to develop a biologically-based large-scale neuromusculoskeletal model intended to investigate the problem of upright stance control from a neurophysiological point of view. Some of its components are: i) conductance-based spinal neuron models (motor neurons and interneurons) arranged in three motor pools (for the Soleus and Gastrocnemii muscles); ii) muscle spindle model providing Ia and II afferent feedback; iii) Hill-type muscle models; iv) inverted pendulum model, which is a first approximation to the body biomechanics during upright posture. The randomness of the system is mainly due to the stochastic point processes that describe the descending neurons' and afferents' activities. The main findings were that the intrinsically unstable mechanical system might be stabilized by appropriately setting the level of fusimotor activity. The resultant values of COP RMS and mean velocity were compatible with those reported from vestibular loss subjects standing on a stable surface without visual information. In addition, there was a small cross-correlation peak at a lag around 300 ms between simulated COP and EMG from the Triceps Surae's muscles. The more interesting finding was that an intermittent pattern of muscle activation emerged from this posture control model, suggesting that the spinal architecture and organization, along with the modulation of afferent activity, may account for the apparent intermittent control that has been postulated by some researchers. From our knowledge this is the first large-scale neuromusculoskeletal model used to control an unstable biomechanical system which approximates human standing. Several other studies may be carried out using this model,

for instance, the investigation on how neuromuscular changes associated with aging or specific diseases influence postural control.

Disclosures: L.A. Elias: None. **R.N. Watanabe:** None. **A.F. Kohn:** None.

Poster

293. **"Computation, Modeling, and Simulation IV"**

Location: Halls B-H

Time: Sunday, November 10, 2013, 1:00 PM - 5:00 PM

Program#/Poster#: 293.08/LLL10

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

Support: NIH Grant 1R21NS075463

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NIH Grant 2R24HD50821

Brinson Stroke Foundation

Davee Research Foundation

Title: Modified motor unit number index: A simulation study

Authors: *P. ZHOU[1,2], X. LI[1], W. RYMER[1], S. NANDEDKAR[3];
[1]Rehabil. Inst. of Chicago, Chicago, IL; [2]Univ. of Sci. and Technol. of China, Hefei, China; [3]Natus Med. Inc, Middleton, WI

Abstract: Motor unit number index (MUNIX) that relies on the compound muscle action potential (CMAP) measurement and different levels of voluntary surface electromyogram (EMG) recordings has recently achieved much attention as a tool for evaluation of neuromuscular disorders. Our previous studies using a motoneuron pool and surface EMG simulation approach have revealed that reduction of individual motor unit action potential (MUAP) amplitude as a result of muscle fiber atrophy leads to substantial underestimation of the motor unit number with the MUNIX measurement. In this study, we introduce a novel concept of modified MUNIX, whose definition is correlated with the CMAP area of the examined muscle. The sensitivity of the modified MUNIX to reduced MUAP amplitude was explored by the motoneuron pool and surface EMG simulation approach. The results indicate that the MUNIX definition with a fixed surface EMG interference pattern area (20 mV•ms) is most suitable for

motoneuron diseases that demonstrate secondary evidence of muscle fiber reinnervation, while the modified MUNIX is applicable to atrophied muscles, where the atrophy is primarily induced from muscle fiber shrinkage rather than motor unit loss.

Disclosures: P. Zhou: None. **X. Li:** None. **W. Rymer:** None. **S. Nandedkar:** None.